Rheumatol Int (1993) 13:139–146

Rheumatology INTERNATIONAL
Clinical and Experimental Investigations

Expression of the components and regulatory proteins of the alternative complement pathway and the membrane attack complex in normal and diseased synovium

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Received April 5, 1993/Accepted July 30, 1993

Summary. We have studied synthesis of the complement components and regulatory proteins of the alternative pathway and the membrane attack complex in synovial membrane. RNA was extracted from synovial tissue of patients with rheumatoid arthritis (RA) or osteoarthritis (OA) as well as from normal synovial membrane. Dot blot analysis showed the presence of mRNAs for all the complement components and regulatory proteins (C3, factor B, factor D, C5, C6, C7, C9, factor H, factor I, S-protein, SP-40, 40, DAF, MCP, CR1, CD59), except for properdin, C8α, C8β and C8γ in all three types of synovial membrane studied. In an attempt to determine which components were synthesised by each cell type, monocytes (mononuclear phagocytes), human umbilical vein endothelial cells (HUVEC), synovial membrane fibroblasts (from normal, OA and RA synovial membrane) and peripheral blood lymphocytes were cultured in vitro and secretion rates of individual components were measured and total cellular RNA analysed by northern blotting. Monocytes secreted properdin, C3, and factor H but not factor B, factor I, C5, C6, C7, C8 or C9. Fibroblasts and endothelial cells secreted factor B, factor H and factor I, but not properdin, C5, C6, C7, C8 or C9. Lymphocytes did not secrete any of these components. mRNAs encoding C3, factor B, factor H, S-protein, SP-40, 40, MCP and DAF were detected in all three other cell types (monocytes, fibroblasts and HUVEC), but factor I and CD59 mRNAs were not detected in monocytes. C5, C6, C7, C8α, C8β, CD8γ and C9 mRNAs were not detected in any of the cell types studied. Cell-specific differences were observed in the expression of the different mRNA species for DAF, MCP and CD59. The results of the present study demonstrate that synthesis of many complement components occurs in normal, RA and OA synovial membrane, and that this may be explained in part by synthesis in mononuclear phagocytes, endothelial cells and fibroblasts. The cellular sources of C5, C6, C7 and C9 mRNAs in synovial membrane have not been determined. The data also show that there are important cell-specific differences in the expression of the genes encoding both the alternative complement pathway components and the membrane regulatory components. These differences require further investigation.

Key words: Complement components – Regulatory proteins – Alternative complements pathway – Membrane attack complex – Synovium

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Introduction

The complement system comprises a group of plasma and associated cell membrane proteins which play a major role in the inflammatory response and in host defence. The system may be activated by either of two pathways, the classical or the alternative. The classical pathway is usually activated by antigen-antibody complexes, whereas activation of the alternative pathway by micro-organism may occur in the absence of antibody (reviewed in [1]). Activation of either pathway results in the formation of multimolecular enzymes which activate the third (C3) and fifth (C5) components, and recruit the components of the terminal sequence to form the C5b-9 cytolytic membrane attack complex (MAC). A number of pro-inflammatory products are generated during complement activation. The anaphylatoxins (C4a, C3a and C5a) are released from the N-termini of C4, C3 and C5 by limited proteolysis during activation of either the classical (C4a, C3a and C5a) or alternative (C3a and C5a) pathways [2]. These peptides activate a variety of inflammatory cells including mast cells, neutrophils and macrophages, they increase vascular permeability and C5a is a powerful chemoattractant (reviewed in [2]). The opsonins C3b and iC3b ligate the complement receptors CR1 and CR3 respectively [3]. Ligation of CR3 on phagocytic cells results in phagocytosis [3]. Assembly of the MAC on non-nucle-

ated cell membranes results in lysis. However, although, nucleated cells are relatively resistant to lysis by the MAC, it assembly on such cells [including neutrophils, macrophages and rheumatoid arthritis (RA) synovial cells] may result in secretion of reactive oxygen metabolites, arachadonic acid metabolites and cytokines (reviewed in [4]). The widespread distribution of the MAC in the tissue lesions of many inflammatory diseases, including RA, in the absence of necrosis provides strong support for a pathogenetic role for the MAC in the cellular activation which occurs in these diseases [4].

RA is a chronic disease affecting principally synovial joints, and is characterised by chronic inflammation and joint destruction [5]. Although antigen-antibody complexes are present within the synovial fluid and joint tissues in association with intense classical pathway activation, significant alternative pathway activation also occurs [6] together with activation of the terminal sequence and MAC assembly [7]. Thus intra-articular complement activation could contribute significantly to the inflammatory process in RA. Although the liver is the primary site of synthesis of most of the plasma complements components (with the exceptions of C1q, factor D and properdin) extrahepatic synthesis of C3 and factor B occurs in most cells, while other complement components are synthesised by cultured mononuclear phagocytes, fibroblasts, endothelial cells, epithelial cells and adipocytes (reviewed in [8]). Whether these cells synthesise complement components in vivo has not been determined. The importance of locally synthesised complement components is unknown: in normal tissues they could play a role in host defence whereas in inflamed tissues they could contribute to the inflammatory process. RA is a chronic inflammatory process in which cellular proliferation/recruitment predominates over exudation and joint destruction often proceeds in the absence of effusion. In addition at the leading edge of the pannus and at the site of erosions, fibroblasts, macrophages and the endothelial cells of the newly formed non-patent capillary buds are present [9], all of which are capable of synthesising complement. Thus, although in acutely inflamed tissues plasma exudation would be expected to provide the major source of complement components in inflammatory exudate, in chronic inflammation and in non-inflamed tissues local synthesis of components might be expected to contribute significantly to the amount in the extravascular fluid. In this context it is important to note that a study of C3 metabolism in a patient with RA showed that approximately half the C3 present in joint fluid had been synthesised locally [10]. In a separate study we have shown that all the components and fluid-phase regulatory components of the classical pathway were synthesised within synovial membrane from normal joints and from joints of patients with RA or osteoarthritis (OA) (submitted for publication) [11]. In this study we have sought to determine (1) which components and regulatory components of the alternative pathway and terminal sequence are synthesised in synovial membrane from patients with RA, OA and in normal synovial membrane, and (2) which cells are capable of synthesising these components.

Materials and methods

Reagents

The following reagents were purchased from the sources shown: Linbro multiwell tissue culture dishes, trypsin-EDTA solution in Puck's saline (trypsin EDTA), Linbro 75 cm^2 tissue culture flasks, Dulbecco's modified Eagle's medium (DMEM), RPMI 1640 (Flow Laboratories, Rickmansworth, England); fetal calf serum (FCS), antibiotic, Hank's balanced salt solution, Nunclon tissue culture flasks 25 cm^2, 75 cm^2; 175 cm^2 (Gibco BRL, Paisley, Scotland); Hybond-N membranes (Amersham International, England); random primed DNA labelling kit, DNase I (RNase free), RNase inhibitor (Boehringer Mannheim, Mannheim, Gemany); diethylpyrocarbonate, gelatin (2% (w/v) solution), endothelial cell growth supplement (Sigma, Poole, England); RNAzol (Biogenesis, Bournemouth, England); human AB serum (ABS) was supplied by the Scottish Blood Transfusion Service (Law Hospital, Carluke, Scotland). FCS and ABS were heat-inactivated (56° C for 2 h) prior to use.

Preparation of cDNA probes

Plasmids containing the following cDNA were used: C3 (PLC351; Dr. G. Fey, Scripps Clinic and Research Foundation, La Jolla, Calif.) [12]; factor B (p2FB; Dr. R. D. Campbell, Oxford, England) [13]; properdin (P516; Dr. K. B. M. Reid, MRC Immunochemistry Unit, Oxford) [14]; factor D (hg 31-40, Dr. T. White, Metabolic System Inc, Mountain View, Calif.) [15]; C5 (C5HG2; Dr. B. Tack, Scripps Clinic and Research Foundation, La Jolla) [16]; C6 (C6α; Dr. M. J. Hobart, MRC Molecular Immunopathology Unit, Cambridge, England) [17]; C7, C9 (HL/C7: 81423, pHL C9/55; Dr. R. DiScipio, Scripps Clinic and Research Foundation, La Jolla) [18, 19]; C8α, C8β, C8γ (Dr. J. M. Sodetz, Department of Chemistry, University of South Carolina, Columbia) [20–22]; factor H, factor I (B38-1, psp64; Dr. R. B. Sim, MRC Immunohistochemistry Unit, Oxford), SP-40,40 [23, 24] (LK 107, Dr. B. Murphy, St. Vincent's Hospital, Fitzroy, Victoria, Australia) [25]; S-protein/vitronectin (Bioquote Ltd, Ilkley, England), membrane cofactor protein (MCP-9; Dr. D. M. Lublin, Washington University School of Medicine, St. Louis, Mo.) [26]; decay accelerating factor (DAF2.1; Dr. M. E. Medof, Department of Pathology, Case Western Reserve University, Cleveland, Ohio) [27]; complement receptor type 1/C3b receptor (pCR1.1; Dr. D. T. Fearson, Department of Medicine, Johns Hopkins University, Baltimore, Md.) [28]; CD59 (YTH53.1/1; Professor H. Waldmann, Department of Pathology, University of Cambridge, England) [29].

After the cDNA inserts had been excised from their vectors with the appropriate restriction endonucleases, they were purified by electrophoresis in low-melting-temperature agarose followed by phenol/chloroform extraction and ethanol precipitation. S-protein cDNA was amplified by PCR using internal primers (5′ > GCG TCG ACA GAT GGC CAG GA < 3′ and 5′ > GCG AAT TCA CCG ACT CAA GAA C < 3′). cDNAs for C6 and C7 were PCR amplified using M13 mp18 primers, and DAF cDNA was amplified using pGEM primers. Primer concentrations were 0.5 μM and all reactions consisted of 25 cycles of denaturation (96° C for 1 min), annealing (42° C for 2 min) and extension (72° C for 2 min). Radiolabelled α^{32}-P-d-CTP cDNA probes were prepared by the random prime reaction as described previously [31].

Tissues and cells

Synovial tissue. Synovial tissue was collected at the time of surgery from the knee joints of three patients with definite or classical RA, three with OA and three from individuals undergoing meniscectomy who did not have any OA or chronic inflammatory joint disease (normal). Immediately after collection the tissue specimens were snap-frozen in liquid nitrogen and RNA was isolated from the

frozen tissue using RNAzol, as described previously [42]. RNA extracted from the tissues was analysed in formaldehyde denaturing gels and the relative abundances of mRNA species determined by dot-blot analysis using 5 μg RNA/blot [42]. Hybridisation reactions, washing and autoradiography were performed as described for northern blots (see below). Blots were standardised by stripping (100° C in 0.1% (w/v) sodium dodecyl sulphate (SDS) for 5 min) and reprobing with the γ^{32}P-ATP unlabelled synthetic oligonucleotide probe (5′>AAC GAT CAG AGT AGT GGT ATT TCA CC<3′) for 28S rRNA [30].

Synovial fibroblasts. Fibroblasts were isolated from synovial membrane as described previously [42]. Cells were cultured (at 37° C in a humidified 5% CO_2/air atmosphere) containing DMEM and 10% FCS. Studies of complement synthesis were undertaken during the third passage.

Endothelial cells. Primary cultures of human umbilical vein endothelial cells (HUVEC) were prepared from freshly collected umbilical cords, and cultured as described previously [43]. The cells were used for experiments during their fourth passage, at which time they were cultured in RPMI 1640 containing 15% FCS.

Monocytes. Human monocytes monolayers were prepared from the buffy coats of blood donations in 24-well Linbro tissue culture plates [31] and the cells cultured in RPMI 1640 containing 10% ABS at 37° C in a humidified 5% CO_2/air atmosphere. After 3 days cells were washed five times and the medium changed to RPMI 1640 containing 20% FCS and the cells incubated under the same conditions for 24 h before any experiments were performed.

Lymphocytes. The non-adherent cells from the mononuclear leucocyte suspensions used for the preparation of monocyte monolayers were washed in RPMI, resuspended to 2.5×10^6 cells/ml in RPMI containing 10% FCS and incubated at 37° C in a humidified 5% CO_2/air atmosphere.

Total cellular RNA and northern blotting

Total cellular RNA was prepared from cells cultured for 7 days, using RNAzol and northern blotting was performed as described previously [31]. Blots were hybridised (incubation with 10^6 cpm/ml hybridisation fluid at 42° C overnight) to the ^{32}P-labelled cDNAs, washed to high stringency [0.1 × SSC containing 0.1% (w/v) SDS] at 65° C and subjected to autoradiography. Autoradiography were scanned using a Joyce-Loebl Chromoscan-3 (Joyce-Loebl, Gateshead, England). An arbitrary value of 1.00 was assigned to the control level of expression. Northern blots were stripped and reprobed with a synthetic oligonucleotide probe for 28S rRNA as described for dot-blot analysis (see above).

Measurements of proteins in culture fluids

A set of each type of cell culture was incubated for 7 days. At days 1, 3, 5 and 7 the entire culture supernatant was replaced and the used medium stored at − 70° C until assayed. On day 7, the cells were washed and adherent cells were detached by trypsinisation. An aliquot was used for determining cell number and the remainder was used for RNA extraction. The concentrations of C3, factor B, properdin, C5, C6, C7, C8, C9, factor H and factor I were determined by ELISA [31]. We have not yet been able to develop sufficiently sensitive ELISA procedures for factor D, S-protein or SP-40, 40.

Statistics

Differences between the mean values of the relative abundances if each species of mRNA in each of type of synovial membrane and between the mean values of protein secretion rates in each cell type were analysed using Student's *t*-test.

Results

Synovial tissue mRNAs

Dot blots of RNA from RA, OA and normal synovial tissue gave positive hybridisation signals for all the mRNA species studied (C3, factor B and D, C5, C6, C7, C9, factors H and I, S-protein, SP-40,40, DAF, MCP, CR1, CD59) with the exceptions of properdin and C8α, C8β and C8γ (Fig. 1). The relative abundance of C3 mRNA in normal synovial membrane was higher than that in OA tissue ($t = 3.46$, $P < 0.05$) but lower than that in RA tissue ($t = 8.33$, $P < 0.001$) (Table 1). The relative abundance of factor B mRNA was increased in RA tissue compared with normal synovial membrane ($t = 3.22$, $P < 0.05$) (Table 1). With these exceptions there were no significant differences between the relative abundances of mRNAs for factor D, C5, C6 and C7 in the three types of tissue (Table 1, 2). Because of the amount of RNA available the relative abundances of the mRNAs for C9 and the fluid-phase regulatory components were determined in only one sample of each type of synovial tissue. The abundances of the mRNAs for factor H and I and possibly CD59 appeared to be higher in RA compared with normal tissue, while that of SP-40,40 was low in OA tissue (Table 1, 2).

Cell mRNAs

Single species of mRNA were observed for C3 (5.1 kb), factor B (2.7 kb), factor D (2.2 kb), properdin (1.6 kb),

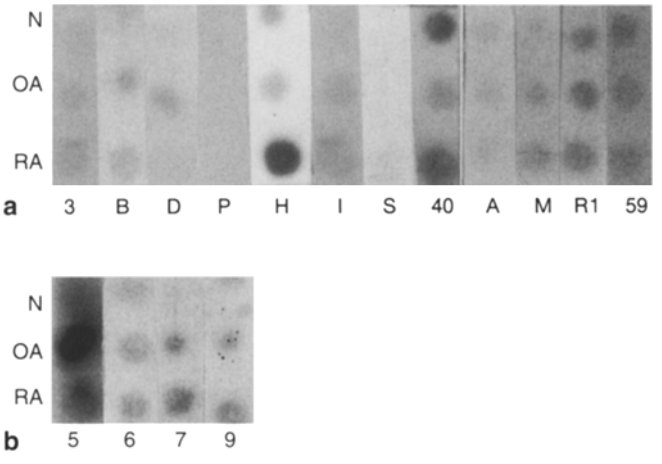

Fig. 1 a, b. Dot blot analysis from normal (*N*), osteoarthritis (*OA*) and rheumatoid arthritis (*RA*) synovial membrane. The dots (5 μg RNA) were probed for **a** mRNAs for C3 (*3*), factor B (*B*), factor D (*D*), properdin (*P*), factor H (*H*), factor I (*I*), S-protein (*S*), SP-40, 40 (*40*), DAF (*A*), MCP (*M*), CR1 (*R1*) and CD59 (*59*) and **b** mRNAs for C5 (*5*), C6 (*6*), C7 (*7*) and C9 (*9*). Properdin and C8 mRNAs were not detected, and the signal for S-protein was very faint

Table 1. Relative abundance of mRNAs for alternative pathway components and control proteins in synovial tissue

Component	Normal	OA	RA
C3	1.00±0.01	0.56±0.22*	1.15±0.03**
Factor B	1.00±0.05	1.45±0.28	1.88±0.47*
Factor D	1.00±0.15	1.33±0.37	1.21±0.21
Factor H[a]	1.00	1.00	2.00
Factor I[a]	1.00	0.7	4.30
MCP	1.00±0.03	0.83±0.1	0.83±0.2
DAF	1.00±1.00	0.4 ±0.3	0.6 ±0.004
CR1	1.00±0.5	1.00±0.2	0.93±0.2

Values are mean±SEM of three experiments
OA, Osteoarthritis; RA, rheumatoid arthritis
[a] Due to shortage of RNA only one value was obtained for the relative abundances of factor H and factor I mRNAs
* $P<0.05$; ** $P<0.001$

Table 2. Relative abundance of mRNAs for terminal sequence components and regulatory proteins in synovial tissue

Component	Normal	OA	RA
C5	1.00±0.15	1.54±0.29	1.28±0.31
C6	1.00	0.98±0.14	1.05±0.21
C7	1.00	1.28±0.28	1.41±0.29
C9[a]	1.00	1.05	1.09
S protein[a]	1.00	3.20	1.50
SP-40,40[a]	1.00	0.30	0.90
CD59[a]	1.00	0.5	2.40

Values are mean±SEM of three experiments
[a] Due to shortage of RNA only one value was obtained for the relative abundance of C9, S protein, SP-40,40 and CD59 in RNAs

factor I (2.4 kb), S-protein (2.8 kb) and SP-40,40 (1.8 kb) whereas multiple bands were observed for factor H (4.3 and 1.8 kb), MCP (4.8 and 4.2 kb), DAF (2.7 and 1.5 kb) and CD59 (2.0, 1.4 and 0.8 kb) performed on northern blots isolated from the cells. CR1 mRNA was not sought in the cell mRNA preparations as it is known to be expressed in monocytes but not in HUVEC or fibroblasts.

Fibroblasts. Messenger RNAs (mRNAs) encoding C3, factor B, factor H, factor I, S-protein, SP-40,40, DAF, MCP and CD59 were detected by northern blotting of RNA prepared from fibroblasts from synovial membrane from all three types of patient. Messenger RNAs for factor D, properdin, C5, C6, C7, C8α, C8β, C8γ and C9 were not detected. The relative abundances of each of the mRNAs were similar in the three types of fibroblast. As the blots for all three types of fibroblasts are essentially the same, we have only shown those blots from fibroblasts from normal synovial membrane (Fig. 2).

Endothelial cells. Messenger RNAs (mRNAs) encoding C3, factor B, factor H, factor I, S-protein, SP-40,40, MCP, DAF and CD59 were detected in endothelial cells (Fig. 2). We were unable to detect mRNAs for properdin, factor D, C5, C6, C7, C8α, C8β, C8γ and C9.

Monocytes. Monocytes expressed mRNAs for C3, factor B, factor D, properdin, factor H, S-protein, SP-40,40,

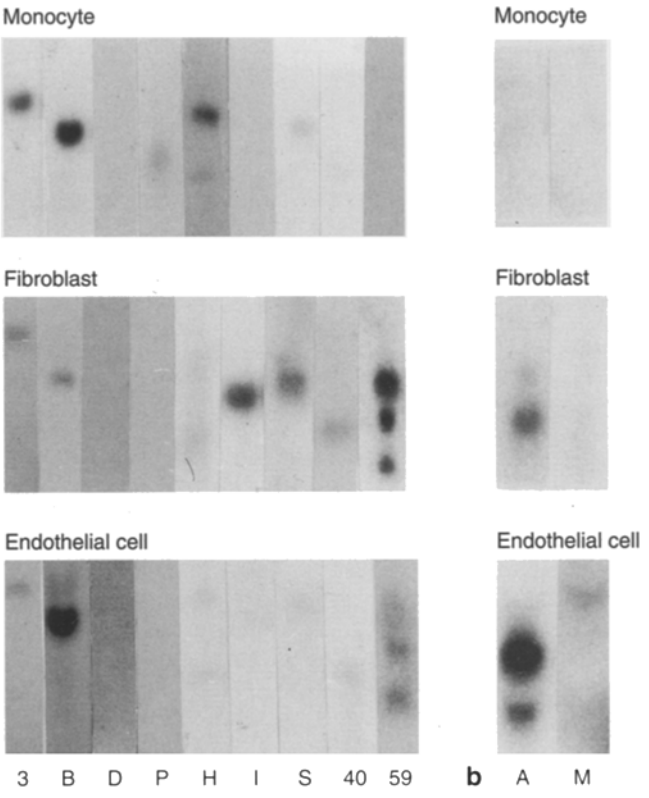

Fig. 2 a, b. Northern blot analysis of RNA from monocytes, synovial fibroblasts (from normal synovial membrane) and human umbilical vein endothelial cells (HUVEC). The blots were probed for **a** C3 (*C*), factor B (*B*), factor D (*D*), properdin (*P*), factor H (*H*), factor I (*I*), S-protein (*S*), SP-40,40 (*40*) and DC59 (*59*) and **b** DAF (*A*) and MCP (*M*). RNA preparations from fibroblast, OA and RA synovial membrane were also analysed but they are not shown as they gave identical results to those from normal synovium. The sizes of the mRNAs were as follows: C3 (5.1 kb), factor B (2.7 kb), factor D (2.2 kb), properdin (1.6 kb), factor H (4.3 kb and 1.8 kb), factor I (2.4 kb), S-protein (2.8 kb), SP-40,40 (1.8 kb), CD59 (2.0 kb, 1.4 kb and 0.8 kb), DAF (2.7 kb and 1.5 kb), MCP (4.8 kb and 4.2 kb)

MCP and DAF, but mRNAs encoding factor I, C5, C6, C7, C8α, C8β, C8γ and C9 were not detected (Fig. 2). Factor H mRNA was only detected when cycloheximide (2.5 μg/ml) was included in the culture medium.

Secretion rates of complement components

The secretion rates of C3, B, P, H and I were linear throughout a 7-day culture period. Monocytes did not secrete factor I, C5, C6, C7, C8 or C9, fibroblasts did not secrete properdin, C5, C6, C7, C8 or C9, endothelial cells did not secrete properdin, C5, C6, C7, C8 or C9 (Table 3). Lymphocytes did not secrete any of the fluid-phase complement components. Factor B secretion was not detected in any of the set of three monocyte cultures (lower limit of sensitivity of ELISA 0.8 ng/ml), despite the observation that factor B mRNA was present. We have previously noted lack of factor B secretion in a significant proportion of unstimulated monocyte cultures. The secretion rate of C3 in monocytes was significantly higher than that for HUVEC ($t=9.81$, $P<0.001$), which in

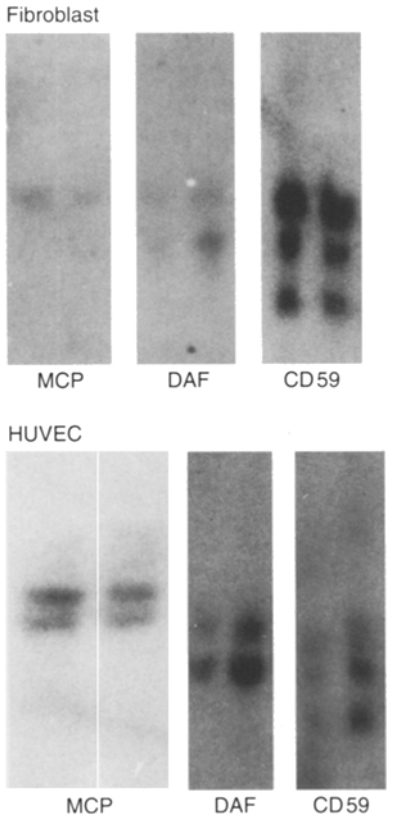

Fig. 3. Northern blot analysis of RNA from normal synovial fibroblasts and human unbilical vein endothelial cells (*HUVEC*). Blots probed for DAF, MCP and CD59 demonstrate cell-specific differences in the expression of different mRNA species. The sizes of the mRNAs were as follows: DAF (2.7 and 1.5 kb), MCP (4.8 and 4.2 kb) and CD59 (2.0, 1.4 and 0.8 kb)

Table 3. Secretion rates of complement components (molecules/min per cell) in different cell types

Component	Monocytes	Fibroblast	HUVEC
Factor B	ND	30±8	26±9
Properdin	561±78	ND	ND
C3	813±71	20±5	110±10
Factor H	65±6	140±22	74±12
Factor I	ND	39±10	40±12

Data for fibroblasts are from normal synovial membrane. The results obtained with fibroblasts from OA and RA synovial membrane were similar. ND, Not detected; HUVEC, human umbilical vein endothelial cells; Data represent the mean±SEM of three experiments. Results of statistical analysis are presented in the text. We were unable to detect C5, C6, C7, C8 or C9 in any of the culture supernatants

turn had a higher rate than fibroblasts ($t = 8.05$, $P < 0.005$). The secretion of factor H in fibroblasts was significantly higher than in monocytes ($t = 3.29$, $P < 0.05$) and HUVEC ($t = 2.63$, $P < 0.05$).

mRNAs for membrane regulatory components

The levels of expression of mRNAs for DAF and MCP in monocytes were too low for accurate densitometric measurement. The presence of mRNAs for DAF, MCP and CD59 in the different cell types was associated with membrane expression of these components as shown by immunohistochemistry and flow cytometry (unpublished data). Although monocytes expressed membrane CD59, we did not detect CD59 mRNA in the cells used in these experiments.

In all three cell types, DAF mRNA resolved into two bands of 2.7 and 1.5 kb. In HUVEC the ratio of the density of the lower band was approximately twice that of the upper band, whereas in fibroblasts the lower band was of equal or lesser intensity than the upper (Table 4).

MCP mRNA was present as a single species (4.2 kb) in fibroblasts (Fig. 3) but resolved into bands (4.8 and 4.2 kb) in HUVEC (Fig. 3). The hybridisation signal for MCP in monocytes (a single band of 4.2 kb) was very weak (data not shown).

CD59 mRNA resolved into three bands (2.0, 1.4 and 0.8 kb) in HUVEC and fibroblasts. The ratio of 1.4/2.0 kb and 0.18/2.0 kb species was greater in HUVEC than in fibroblasts (Table 4). As a control the ratio of 1.8/4.3 kb mRNAs for factor H was measured and shown to be similar in both cell types (Table 4).

Discussion

We have studied the synthesis of the complement components of the alternative pathway (C3, factor B, factor D and properdin), the membrane attack complex (C5, C6, C7, C8, C9) and the regulatory components (factor H, factor I, S-protein and SP-40,40, DAF, MCP and CD59) by synovial membrane. In addition we have also studied the synthesis of these components by mononuclear phagocytes, fibroblasts, endothelial cells and lymphocytes in an attempt to determine which cells in synovial membrane may be responsible for their synthesis. Our results show that the mRNAs for all of these components (except those for properdin, C8α, C8β and C8γ) were present in normal, OA and RA synovial membrane. We

Table 4. Ratios of different species of DAF and CD59 mRNAs in HUVEC and synovial membrane fibroblasts

Component	Ratio	HUVEC	Fibroblast		
			Normal	OA	RA
DAF	1.5 kb/2.7 kb	2.0±1.0	0.80±0.2	0.73±0.2	0.90±0.6
CD59	1.4 kb/2.0 kb	1.17±0.3	0.65±0.1	0.78±0.5	0.78±0.2
	0.8 kb/2.0 kb	1.35±0.6	0.77±0.3	0.90±0.9	0.60±0.5
Factor H	1.8/4.3	0.41±0.14	0.52±0.2	0.58±0.2	0.56±0.2

have not used the polymerase chain reaction (PCR) to amplify the mRNAs encoding C8 and properdin. This extremely sensitive technique has been used to detect C8β in monocytes [32] and should be applied to synovial membrane mRNA before considering that C8 and properdin are not synthesised in synovium. The synthesis of the components of the alternative pathway and the MAC could be important in host defence within the joint, but also could play a significant role in the pathogenesis of the inflammatory response. The increased expression for C3 mRNA and factor B mRNA synovium suggests increased production of these alternative pathway components compared with normal tissue. The reduction in C3 mRNA abundance in OA synovium could also be important but could represent a sampling problem as the synovial changes of OA are often focal in nature. The single determinations for factor H and factor I mRNA suggest that synthesis of these fluid-phase alternative pathway control proteins may be increased in RA synovium. These two components (factor H and I) prevent inappropriate fluid-phase C3 activation which is essential for efficient deposition of C3 on activating surfaces. Unfortunately we were unable to measure confidently synthesis of the fluid-phase components by synovial tissue fragments cultured in vitro as we could not suppress consistently the release of proteins from the tissue fragments with cycloheximide (2.5 μg/ml) (data not shown). Thus the majority of proteins accumulating in the tissue culture medium could have resulted from plasma contamination. In a previous study [33] we were able to suppress synthesis of C1-inhibitor, C4, C2, C3 and factor B with cycloheximide. We cannot account for the discrepancy, but minor differences in sample preparation and washing are probably responsible.

Normal synovial membrane contains a variety of cells including type A (macrophage-like) and B (fibroblast-like) synoviocytes, fibroblasts, macrophages, endothelial cells and adipocytes [5]. In OA, hyperplasia of the synovial lining layer occurs in association with focal lymphoid aggregates, while in RA hyperplasia of the lining layer is associated with chronic inflammation characterised by infiltration of lymphocytes, plasma cells, macrophages, fibroblasts and endothelial cells [34]. Thus in normal synovium and in RA and OA synovial membrane there is a variety of cells which are capable of synthesising complement components. Previous studies have shown that monocytes synthesise C3, factor B, factor D, properdin, factor H, factor I, DAF, MCP, CR1 and CD59; fibroblasts synthesise C3, factor B, factor H, DAF and MCP; endothelial cells synthesise C3, factor B, factor H, factor I, DAF, MCP, CR1 and CD59, and most lymphocytes synthesise DAF, MCP, CR1 and CD59 (reviewed in [8]). Our own immunohistochemical studies (unpublished data) agree with the data on the distribution of DAF, MCP, CR1 and CD59 on these cells and have shown that most cells in normal, OA and RA synovial membrane express DAF, MCP and CD59.

Although monocytes expressed factor B mRNA, factor B protein was not detected in the culture supernatants in these experiments. It has been noted previously that the secretion rate of factor B in unstimulated monocytes may be extremely low or absent [8]. This failure of secretion of factor B in the presence of factor B mRNA suggests that translation of factor B may be inefficient in unstimulated monocytes. In contrast, in the presence of interferons α/β or γ synthesis is increased dramatically, although it appears that the majority of this increased synthesis is related to increased the transcription of factor B gene [35]. We do not know whether interferons or other cytokines which are synthesised in synovial membrane are able to regulate factor B synthesis at the translational level.

Previously the ability of monocyte conditioned media to inactivate C3b was taken as evidence of factor I synthesis [36], but our failure to demonstrate factor I protein and or factor I mRNA suggest that C3b inactivation was due to a protease(s) other than factor I. Synthesis of factor I by fibroblasts has not been reported previously. Another discrepancy was the finding of properdin synthesis by cultured monocytes while properdin mRNA was not detected in synovial membrane RNA preparations. As mononuclear phagocytes and lymphoblastoid cell lines [36, 37] appear to be the only cells which express properdin mRNA, it is likely that properdin is synthesised in synovial membrane, but perhaps in this environment the mRNA is unstable.

Although we detected mRNAs for C5, C6, C7 and C9 in synovial tissue RNA, we were unable to detect these mRNAs for corresponding proteins in any of the cells studied. Hetland et al. [38] detected all five terminal components in monocyte and fibroblast cultures and Reed et al. [39] found evidence of C5 synthesis in lymphoblastoid cell lines. We are unable to explain these differences but the lower limits of detection of our ELISA procedures for C5–C9 components ($< 50-100$ pg/ml) may be too insensitive. A previous study has shown that C8β mRNA was expressed in cultured monocytes using PCR [32]. It is possible that these mRNAs and proteins are expressed at a very low level in these cultured cells, or, they are expressed in vivo, but the cells lose this ability when cultured in vitro. Alternatively cells other than those studied may synthesise them. To our knowledge this is the first time that synthesis of SP-40,40 by monocytes, HUVEC and fibroblasts and expression of CD59 by fibroblasts has been demonstrated. Although cells in culture in vitro synthesise complement components, they may not do so in vivo. Likewise cells which synthesise complement components in vivo may loose this property in vivo. Thus in situ hybridisation histochemistry studies must be undertaken to identify which components are synthesised by each cell type in normal, RA and OA synovial membrane. This is particularly relevant in determining which components are synthesised by endothelial cells as HUVEC may not be representative of the vascular endothelium in general.

Although it appears that all the components of the classical [33] and alternative pathways and the terminal sequence (with the possible exception of properdin and C8) are synthesised in synovial membrane, the importance of synovial membrane synthesis has yet to be established. It has been shown that half of the C3 present in an RA joint is synthesised locally [10]. If this level of contri-

bution is true for all components and as depletion of alternative pathway and terminal components occurs in RA synovial fluid [7], then local synthesis must contribute to inflammatory synovitis. Local synthesis of complement in normal synovium may also be important in host defence or in the regulation of cellular activity within the joint tissues.

The data presented in this paper also show that some complement components are not synthesised by all cells (e.g. properdin, factor I) or the rates of synthesis are not the same in the different cell types (e.g. C3). These examples of cell-specific regulation require investigation, as does the regulation of the expression of these genes by the cytokines which are produced within synovial membrane [40]. It is also apparent that in order to generate an efficient alternative pathway C3 convertase from locally synthesised components, monocytes and another cell type must be present in order to synthesise all the components including the fluid-phase regulatory components which prevent inappropriate C3 turnover.

The widespread cell distribution of DAF, MCP and CD59 in different cell types is expected in view of their importance in protecting cells from complement-mediated attack. Cell-specific differences in the expression of the different mRNA species for DAF, MCP and CD59 were also demonstrated. These differences are probably due to cell-specific differences in mRNA processing. MCP has been shown for the tissue-specific polymorphism of MCP which is due to differential splicing [41]. Whether the tissue specific differences in the expression of the different mRNA species for DAF, MCP and CD59 is associated with differences in the levels of expression or activity of the protein products remains to be determined.

Acknowledgments. This study was supported by grants from the Scottish Home and Health Department and the Arthritis and Rheumatism Council. Dr. Lemercier is in receipt of an EEC fellowship.

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