Possible clearance of effete polymorphonuclear leucocytes from synovial fluid by cytophagocytic mononuclear cells: implications for pathogenesis and chronicity in inflammatory arthritis

S T M Jones, J Denton, P J L Holt, A J Freemont

Abstract

A feature common to all forms of chronic inflammatory arthritis, irrespective of the possible underlying cause, is the persistent exudation of large numbers of polymorphonuclear leucocytes (PMNL) into synovial fluid. These cells possess potent degradative enzymes and proinflammatory mediators, and their removal is vital to normal inflammatory resolution. A major route of disposal of extravasated PMNL appears to be programmed cell death (apoptosis), followed by their rapid recognition, and intact phagocytosis, by mature tissue macrophages. Such macrophages, containing PMNL (cytophagocytic mononuclear cells (CPM)), long recognised in synovial fluid as Reiter cells, are commonly found in reactive arthritis, spondyloarthritis, and crystal arthritides, but only rarely in rheumatoid disease.

In a retrospective analysis of 187 knee synovial fluid cytospins, the relation between the formation of CPM and the presence of apoptotic (pyknotic) PMNL was investigated. As long as the synovial fluid examined was fresh there was a high correlation between numbers of CPM (as a percentage of macrophages) and pyknotic numbers of PMNL in fluids containing CPM. This suggests that the formation of CPM occurs in vivo and is involved in the disposal of PMNL. Numbers of pyknotic PMNL increased rapidly in stored synovial fluid without a significant change in numbers of CPM, and were highest in synovial fluid which did not contain CPM. The presence or absence of CPM, or their disease associations, could not be explained simply by limiting numbers of macrophages, or apoptotic PMNL in synovial fluid. These findings are consistent with a regulatory role for CPM in synovial fluid, where they may be important in preventing autolysis of PMNL, and thus local tissue damage.

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Departments of Rheumatology and Pathology, Manchester Royal Infirmary and Manchester Medical School, Oxford Road, Manchester M13 9WL, United Kingdom
S T M Jones
J Denton
P J L Holt
A J Freemont

Correspondence to:
Dr Jones.

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The hallmark of inflammatory arthritis, from a variety of seemingly unrelated causes, is the early and continued accumulation of large numbers of polymorphonuclear leucocytes (PMNL) in synovial fluid.[1] Often dismissed as of secondary importance to events in the synovial membrane, it is generally accepted that numbers of PMNL in synovial fluid most accurately predict clinical indices of local inflammation and disease activity in arthritis[1] and may play a pivotal part in the coordination of later inflammatory processes by their release of strongly chemotactic products, particularly for macrophages.[2] Polymorphonuclear leucocytes are a major source of potent oxidative and proteolytic lysosomal enzymes found in synovial fluid,[3–5] including a specific elastase and an active metalloproteinase[6 7] capable of degrading intact cartilage,[8] as well as isolated extracellular matrix components.[6 9] Massive release of these enzymes, such as in septic arthritis,[4] may saturate their complex inhibitor systems and lead to rapid joint destruction; in the rheumatoid joint surface adherent PMNL may attack the cartilage directly.[10]

It has been realised that extravasated PMNL undergo time dependent programmed cell death (apoptosis),[11] a stereotyped sequence of biochemical and morphological changes, which apears to be an essential and rate limiting step in their recognition, followed by intact phagocytosis by mature tissue macrophages,[12 13] forming cells histologically recognisable as cytophagocytic mononuclear cells (CPM).[14] These are large cells of macrophage morphology containing intracytoplasmic inclusions, either complete PMNL or recognisable cell nuclei.

Such CPM have been recognised histologically for a number of years in pathological specimens of bone marrow,[15] pleural and peritoneal fluids,[16] and also in inflammatory synovial fluid where they are known as Reiter/Pekin cells.[1 17–19] Unlike most synovial fluid leucocytes which do not differentiate between subgroups of arthritides,[20] CPM are only commonly found in certain disease states; though once thought to be specific for Reiter's disease,[21] where they are found in 50–70% of synovial fluid specimens, they are also recognised in other seronegative spondyloarthropathies (40%), in crystal arthropathies (20–30%), and much less often in rheumatoid arthritis.[22]

In the light of these observations we have studied the occurrence of apoptosis of PMNL and its possible bearing on the formation of CPM (Reiter cells) in a cytological survey of knee synovial fluid cytospins, to see whether the suggestion that the formation of CPM depends only on the presence of apoptotic PMNL holds true in synovial fluid, and whether the differential association of CPM with certain clinical subsets relates to known factors limiting their formation.

Materials and methods

Synovial fluid specimens were obtained for

routine diagnostic purposes from a cross section of inpatients and outpatients presenting with joint effusions, and were analysed at one centre (Manchester Medical School). Specimens were either processed for cytological examination within two hours of arthrocentesis ('fresh') or were stored at 4°C until used, at most until the next day ('delayed'), as is normal laboratory practice. To obtain sufficient numbers for quantitative analyses, all synovial fluid samples containing CPM (1987–90) from two centres (Manchester Royal Infirmary, University Hospital of South Manchester) were identified from a database of results; a second series of all synovial fluid samples obtained between 1989 and 1990 from Manchester Royal Infirmary alone was used to calculate frequencies (table 2), and to identify negative controls for CPM. Although only knee fluids were examined, patients were otherwise unmatched for age, sex, or treatment at the time of joint aspiration.

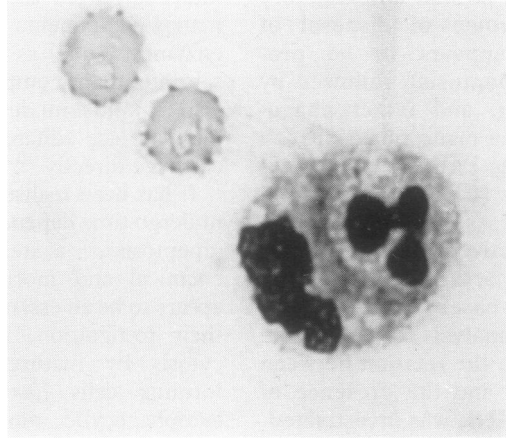

Figure 1 Cytophagocytic mononuclear cell: well preserved apoptotic polymorphonuclear leucocyte within macrophage. Jenner-Giemsa; crenated red blood cell=6 μm.

Table 1 Numbers of synovial fluid samples not suitable for analysis. Seventy one synovial fluid samples used were positive for cytophagocytic mononuclear cells and 116 were negative

Reason for exclusion	*No of samples*
Synovial fluid slide lost/destained	26
Synovial fluid heavily contaminated by blood	15
Case notes not found	7

A count of the total number of leucocytes in synovial fluid was first obtained using a haemocytometer, and standard density cell monolayers were prepared by cytocentrifugation. The monolayers were fixed in methanol, stained by a conventional Jenner-Giemsa technique and examined at 400×magnification. A differential count (PMNL, small lymphocytes, monocytoid cells) was performed on the first 500 cells; the percentage of apoptotic PMNL (recognised by their characteristic nuclear pyknosis, cytoplasmic vacuolation, and loss of bridging chromatin[11] (fig 1)) was expressed as the proportion of the first 200 PMNL. The percentage of CPM (cells with monocytoid morphology containing basophilic intracytoplasmic phagocytosed cell nuclear material[21]) was calculated as a proportion of the first 50–100 monocytoid cells; if the number of cells was less than this (typically <20/slide), a differential count of CPM was not performed, and the results were simply noted as 'too few for analysis'.

As a result of the rapid breakdown of ingested material once phagocytosed, it was not always possible to confirm histologically that the ingested material within the CPM was from PMNL, though the fact that occasional intact phagocytosed PMNL, but not other cell types, were seen was in agreement with PMNL being the main source. The number of degenerate cells (nuclear ghosts) was also recorded as a proportion of the first 200 nucleate cells, giving an index of non-viability of synovial fluid; signs of impending cell death (ballooning, clumping, loss of membrane integrity) were also noted.

All fluids were assessed 'blind' to clinical details, and counts of CPM and apoptotic PMNL used in the analysis were performed by different investigators (JD and SJ respectively). The counts of CPM and apoptotic PMNL were reproducible, with interobserver and intraobserver variations of <5% (data not shown).

Accurate clinical diagnosis using standard American Rheumatism Association criteria, duration of index joint flare, and any delay in synovial fluid processing (between joint aspiration and registration in the laboratory) were obtained by medical case note review.

Statistical analysis was by χ^2, Student's t tests, and Pearson correlation coefficients performed by the SPSS package, where appropriate.

Table 2 Prospective analysis of cytophagocytic mononuclear cell (CPM) positivity by disease group. All synovial fluid obtained in 1989–90, Manchester Royal Infirmary alone

	Total	*No (percentage) CPMs positive*
Osteoarthritis	3	0 (0)
Traumatic effusion	4	0 (0)
Juvenile arthritis	5	0 (0)
Systemic lupus erythematosus	2	0 (0)
Seronegative rheumatoid arthritis	15	1 (7)
Seropositive rheumatoid arthritis	68	9 (13)
Pseudogout	6	1 (17)
Spondyloarthritis*	11	6 (54)
Reiter's disease	11	6 (54)
Septic arthritis	10	6 (60)
Unclassified†	15	9 (60)
Gout	8	5 (63)
Reactive arthritis	4	3 (75)

*Ankylosing spondylitis (n=2), psoriatic arthritis (n=6), and inflammatory bowel disease (n=3).
†Other sheep cell agglutination titre, antinuclear factor negative inflammatory arthritides.

Results

DISEASE ASSOCIATIONS

One hundred and eighty seven synovial fluid samples with adequate clinical details were suitable for analysis: 71 containing CPM and 116 with no CPM (table 1). In accordance with previous data,[22] CPM were seen in 75% of samples from patients with reactive arthritis; approximately 60% of synovial fluid samples from patients with septic arthritis, spondyloarthritis, Reiter's syndrome, and gout; 13% of patients with seropositive and 7% of patients with seronegative rheumatoid arthritis, but not in synovial fluid samples from patients with non-inflammatory arthritis, systemic lupus erythematosus, or juvenile arthritis (table 2).

Table 3 Comparison of synovial fluid cytology between disease groups typically positive and typically negative for cytophagocytic mononuclear cells (CPM). Values are mean (SD) percentage of cells

Type of cell	*All disease groups (n=187)*	*Rheumatoid arthritis (n=73)*	*Diseases typically positive for CPM (n=53)*
Polymorphonuclear leucocytes (PMNL)	63 (26·3)	64·3 (28·6)	62·2 (22·7)
Apoptotic PMNL	11·9 (14·6)	12·1 (16·4)	10·7 (13·0)
Monocytes	10·9 (14·7)	8·0 (9·3)†	12·9 (16·0)†
Ghost	4·4 (5·2)	5·0 (5·4)	3·9 (4·7)

*All negative for CPM.
†p=0·07; otherwise NS.
‡Spondyloarthritis, gout, septic arthritis, and reactive arthritis or Reiter's syndrome.

Table 4 Comparison of synovial fluid cytology between all fluids positive and negative for cytophagocytic mononuclear cells (CPM). Values are mean (SD) percentage of cells

Type of cell	*Synovial fluid positive for CPM (n=71)*	*Synovial fluid negative for CPM (n=116)*	*p Value*
Polymorphonuclear leucocytes (PMNL)	62·2 (22·4)	63·4 (30·2)	NS
Apoptotic PMNL	11·8 (13·0)	12·0 (15·5)	NS
Monocytes	16·0 (18·0)	7·8 (11·4)	<0·01
Ghost	2·7 (3·7)	5·5 (5·7)	<0·001

Unfortunately, samples from patients with non-inflammatory arthritis were scarce in this series because most of the specimens received had too high a viscosity and too few cells for routine diagnostic cytospin preparation. Apart from this problem, the samples studied were a fair representation of normal laboratory throughput.

To see whether these differences in the number of CPM between the various arthritides depended only on the presence of sufficient macrophages and apoptotic PMNL to interact in the synovial fluid, the numbers of these cells were compared between samples from patients with arthritis typically positive for CPM (combined reactive arthritis, spondyloarthritis, crystal arthritis, and septic arthritis) and (rheumatoid) arthritides typically negative for CPM (table 3). In addition, all synovial fluid samples positive for CPM were compared directly with fluids negative for CPM (table 4).

Apoptotic PMNL comprised 11·9 (SEM 14·6)% of the total PMNL overall, and no significant difference was seen either in the total number of PMNL or the percentage of apoptotic PMNL between disease subsets or between fluids positive or negative for CPM. Similar results were obtained either when the individual diseases positive for CPM were considered separately, or when cell counts were expressed in absolute numbers (data not shown). There was a trend towards higher percentages of monocytes in synovial fluid in diseases typically positive for CPM, which became highly significant when fluids positive for CPM were compared directly with fluids negative for CPM (table 4).

Interestingly, the number of degenerate 'ghost' cells was significantly lower in the fluids positive for CPM, and showed no relation with proportions of other cell types. This suggests that macrophages forming CPM may actively remove 'ghost' cells in addition to apoptotic PMNL, making it unlikely that degenerate cells *per se* were an important stimulus to the formation of CPM. A comparison between fluids negative and positive for CPM from non-rheumatoid sources (table 5) showed considerably fewer monocytes and more apoptotic PMNL in the former group, suggesting that quantitative and possibly qualitative differences in monocyte function were responsible for the formation of CPM, and that the formation of CPM may be important in the removal of apoptotic PMNL in these disease groups.

Table 5 Synovial fluid cytology of fluids positive and negative for cytophagocytic mononuclear cells (CPM) in subjects with reactive arthritis, Reiter's syndrome, spondyloarthritis, crystal, and septic arthritides. Values are mean (SD) percentage of cells

Type of cell	*Synovial fluid positive for CPM (n=31)*	*Synovial fluid negative for CPM (n=22)*	*p Value*
White blood cells	17·1 (21·6)	14·2 (19·3)	NS
Polymorphonuclear leucocytes (PMNL)	65·7 (22·9)	65·1 (30·8)	NS
Monocytes	15·3 (17·2)	6·5 (10·8)	<0·01
Apoptotic PMNL	7·9 (7·3)	13·8 (18·2)	<0·02

Table 6 Pearson correlation coefficients (r) between percentage of apoptotic polymorphonuclear leucocytes (PMNL) and other nucleated cells in synovial fluid

Type of cell	*Synovial fluid received same day (n=126)*	*Synovial fluid received next day (n=61)*	*All samples (n=187)*
White blood cells	−0·07	−0·16	−0·11
PMNL	−0·25*	−0·24	−0·20*
Lymphocytes	0·09	0·27	0·13
Monocytes	0·37**	0·25	0·26**

*p<0·05; **p<0·01.

Table 7 Pearson correlation coefficients (r) between percentage of ghost cells and other cell types in synovial fluid

Type of cell	*Synovial fluid received same day (n=126)*	*Synovial fluid received next day (n=61)*	*All samples (n=187)*
White blood cells	0·01	0·06	0·001
Polymorphonuclear leucocytes (PMNL)	−0·13	0·15	0·001
Lymphocytes	0·15	−0·18	0·01
Monocytes	−0·05	−0·07	−0·07
Apoptotic PMNL	0·32**	0·19	0·28*

*p<0·05; **p<0·01.

CORRELATIONS BETWEEN CELL TYPES

To assess the possible origin of ghost cells and any interactions between apoptotic PMNL and other cell types, correlation coefficients were derived between the percentage of apoptotic PMNL (as a proportion of the total number of PMNL), the percentage of ghost cells, and the percentage of other cell types (as differential leucocyte count (tables 6 and 7)). All correlations were performed relative to the storage times of

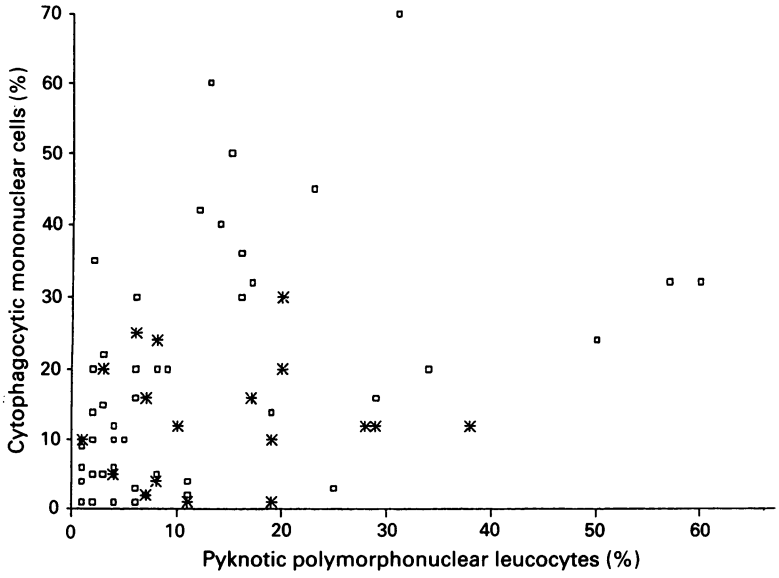

Figure 2 Scattergram showing percentage cytophagocytic mononuclear cells v pyknotic polymorphonuclear leucocytes for fresh (□) and one day old (n=13) or recently injected (n=5) synovial fluid (*).

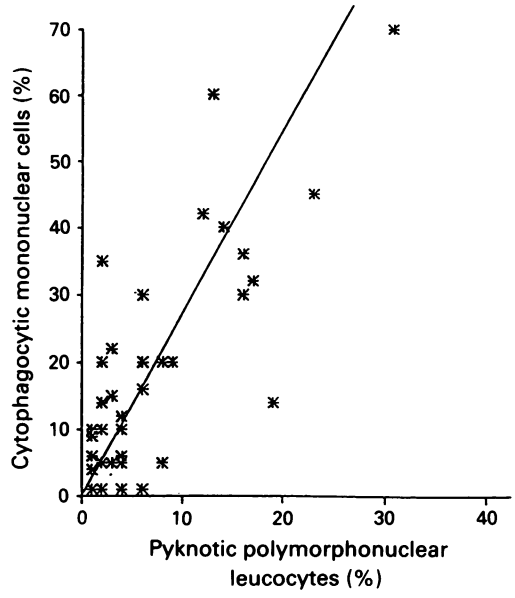

Figure 3 Scattergram showing percentage of cytophagocytic mononuclear cells v pyknotic polymorphonuclear leucocytes for all non-rheumatoid synovial fluid received the same day (r=0·78; p<0·01; n=36).

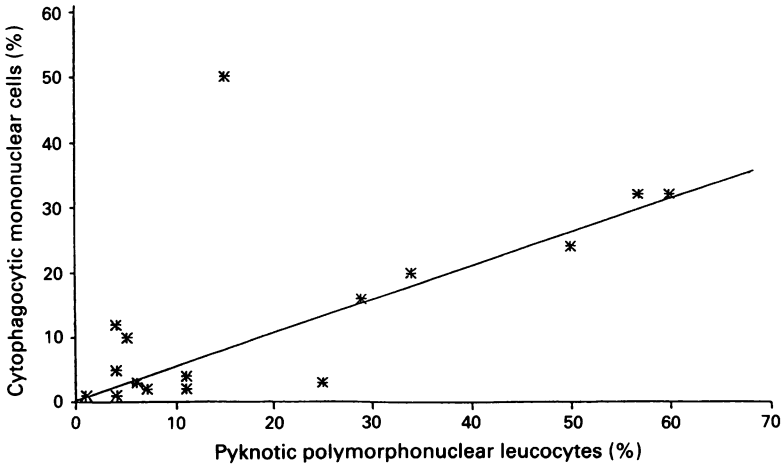

Figure 4 Scattergram showing percentage of cytophagocytic mononuclear cells v pyknotic polymorphonuclear leucocytes for all rheumatoid arthritis synovial fluid received the same day (r=0·61; p<0·01; n=16).

the synovial fluid before processing to try to separate in vitro from probable in vivo effects. The association between CPM and apoptotic PMNL is considered separately in the following.

There was a significant but weak negative correlation between the percentage of PMNL and the percentage of apoptotic PMNL, and a strongly positive correlation between the percentage of monocytes and the percentage of apoptotic PMNL in fresh synovial fluid. These correlations became weaker and statistically insignificant in stored synovial fluid, suggesting that apoptotic PMNL may provide a chemotactic signal for macrophage entry into synovial fluid. The negative association between the number of PMNL and apoptosis, given the lack of association between the presence of CPM and the percentage of PMNL (table 4), could be explained by the recurrent ingress of non-apoptotic PMNL into inflamed joints, leading to higher counts of PMNL and only later the intra-articular development of apoptosis.

The only positive association with ghost cell numbers (table 7) was with apoptotic PMNL in fresh synovial fluid, making these a likely source for the ghosts. This also agrees with the observation that ghost cell numbers were considerably reduced in fluids forming CPM. In stored synovial fluid this association was less marked, possibly via a contribution by non-apoptotic PMNL.

CORRELATION BETWEEN APOPTOTIC PMNL AND FORMATION OF CPM

Work in vitro using aged peripheral blood PMNL and macrophage monolayers has shown a close correlation (r>0·80) between the percentage of apoptotic PMNL and the percentage of CPM.[12] These two variables, derived from fluids positive for CPM only, were plotted in scattergram format (figs 2–4) and correlation coefficients were derived (table 8) within subgroups of conditions known or thought to influence this interaction[12] (time ex vivo; duration of exacerbation in aspirated joint; signs of synovial fluid degeneration, as seen by greater than 50% of nucleate cells showing signs of cell death; recent (within three months) intra-articular steroid injection; and individual diseases).

From an original correlation of +0·44, grouping according to any duration of synovitis, high degeneration scores, recent injection, or processing delay greater than 16 hours detracted from the association. If the synovial fluid sample was processed the same working day, a highly significant correlation was obtained (r=0·78; p<0·01) for all fluids other than those from patients with rheumatoid arthritis, and to a similar, though lesser extent in rheumatoid arthritis (r=0·61; p<0·05). Further subdivision into individual diseases produced no additional improvement in correlation coefficients (data not shown). It should be made clear that these correlations hold only in those synovial fluid samples in which CPM were found, most synovial fluid samples possessing similar numbers of apoptotic PMNL but no CPM (tables 3 and 4).

Discussion

These results provide evidence that apoptosis of PMNL may be necessary for the formation of CPM in synovial fluid, as has been predicted from experimental models of acute inflammation. The differential distribution of CPM between different disease subsets cannot simply be explained by limiting numbers of apoptotic PMNL or of macrophages, however, though there were significantly fewer of the latter in synovial fluid where there was an unexpected absence of formation of CPM. As this was a purely morphological study, it does not exclude differences in the maturity of PMNL or macrophages[14] or in activation status,[23 24] which could have affected the formation of CPM.

Although the initiation of inflammatory synovitis is becoming increasingly well understood, little is known about the mechanisms governing the resolution of normal acute inflammation, defects which could be equally important in the generation of chronic arthritis. Once they have left the bloodstream, there is little evidence that PMNL are able to recirculate as do lymphocytes; they appear to accumulate at sites of inflammation. It has previously been assumed that such extravasated PMNL undergo autolysis in situ, releasing their proinflammatory and histotoxic contents,[25] and so the removal of these cells is likely to be essential to any reparative process.

The rapid, numerically important clearance of extravascular PMNL by the processes of apoptosis and macrophage engulfment may be a major mechanism in this process.[26] Of special importance in the context of inflammatory resolution, such engulfment does not appear to lead to the activation of either cell, or to lysosomal enzyme release, and therefore may represent an important common pathway both for the resolution of synovitis and possibly the limitation of local tissue damage.[12]

In rodent models such as glycogen or streptococcal induced peritonitis[26 27] large numbers (greater than 20% of cells) of CPM are seen in peritoneal lavage fluids at 48 hours after injection, coexistent with the disappearance of PMNL. The number of CPM increase in response to the intensity of the inflammatory response as gauged by a higher, earlier peak of PMNL, or if bacteria are used, leading to their phagocytosis and cell activation. Similarly, higher counts of CPM were also seen after second challenges, when there was already a resident population of active macrophages; the latter is probably a better approximation to the situation in chronic synovitis, where synovial fluid macrophages typically bear activation antigens.[24] Experiments using human peripheral blood mononuclear cells show that these have to be cultured for two to seven days before the formation of CPM is possible, coinciding with their differentiation into mature macrophages.[14]

Although it might be predicted from this that a better correlation between CPM and apoptotic polymorphs may occur in recent onset synovitis, this was not so (table 8). The loss of the physiological association between these cell numbers in stored synovial fluid, and the wide variation in the percentage of apoptotic PMNL in recently aspirated fluids, all point to the cell interaction occurring mainly in situ in the joint, rather than as an artefact after aspiration. Polymorphonuclear leucocytes in vitro, and in stored synovial fluid, tend to age in phase, with a maximum viability of 24–48 hours, during which time an increasing proportion becomes apoptotic.[12] Most aspirated joints had been inflamed for more than one week, and sometimes for several months (table 9), suggesting the continued recruitment of new PMNL into the joint. This is supported by labelling experiments which have shown a half life for intra-articular PMNL of approximately six hours.[28] As the influx of PMNL is probably continuous after the onset of synovitis, its duration per se is unlikely to have any major effect on the ratio of apoptotic PMNL to CPM.

Apoptosis (programmed cell death) has long been recognised as an important trigger to target cell recognition and disposal of phagocytes, in the processes of tissue involution and remodelling, where there is typically no residual tissue damage.[11] Its role in the control of the immune response is only now being recognised; it may be crucial in T cell clonal deletion and the generation of self tolerance in the developing thymus.[29] It has also been implicated in natural killer cell mediated cytolysis and tumour necrosis factor,[30] and now, as discussed here, in the removal of PMNL from inflamed tissues.

Apoptosis in PMNL, as in other cell types, is associated with the progressive appearance of low molecular mass DNA fragments, consistent with the activation of an internucleosomal endonuclease, and typical morphological changes, including chromatin aggregation, nuclear pyknosis, and cytoplasmic vacuolation. Membrane and organelle structures are preserved until late in the process, which is distinct

Table 8 Pearson correlation coefficients between percentage cytophagocytic mononuclear cells (CPM) and percentage apoptotic polymorphonuclear cells within specified subgroups of fluids positive for CPM

Characteristics	r Value	p Value	
Normal cells (>50%) (n=50)	0·02	NS	Decreasing correlation
Synovial fluid late at laboratory (n=18)*	0·03	NS	
Sick cells (>50%) (n=16)	0·06	NS	
Duration of synovitis (days) (n=28)	0·16	NS	
Duration of synovitis (weeks) (n=20)	0·25	NS	
Duration of synovitis (months) (n=18)	0·31	NS	
All samples (baseline) (n=71)	0·44	<0·01	Increasing correlation
Synovial fluid received same day (n=53)	0·46	<0·01	
Diagnosis of RA, same day† (n=16)	0·61	<0·05	
Diagnosis not RA (same and late) (n=48)	0·63	<0·01	
Diagnosis not RA, same day (n=36)	0·78	<0·01	

*Includes five synovial fluid samples from recently injected joints, number too small for meaningful separate statistical analysis.
†RA=rheumatoid arthritis.

Table 9 Duration of synovitis at the time of arthrocentesis

Duration	No (%) of samples
Less than one week	39 (21)
One week–one month	35 (19)
More than one month	113 (60)

Table 10 Effect of RPMI 1640 dilution on formation of cytophagocytic mononuclear cells (CPM) and apoptotic polymorphonuclear leucocyte (Apo PMNL) clearance in short term synovial fluid culture (16 hours, 37°C, 5% CO_2). Values are mean (SEM) percentages

Synovial fluid concentration (%)	*CPM (A) (%)*	*ApoPMNL (A) (%)*	*CPM (B) (%)*	*ApoPMNL (B) (%)*
100	21·4 (7·7)*	32·4 (15·9)	0	63·3 (14·5)
50	7·0 (1·8)	53·0 (18·1)	0	70·3 (15·3)
25	4·6 (1·7)	59·2 (15·6)	0	66·7 (12·2)
12·5	2·6 (1·6)	76·0 (10·1)	0	72·0 (14·4)
p Value, 12·5% *v* 100%	0·07	0·05	NS	NS

*A, Synovial fluid producing CPM (n=6) (spondylo/reactive arthritis); B, synovial fluid not producing CPM (n=5) (all rheumatoid arthritis).

from autolysis/necrosis.[13] In this study, these typical appearances were used as markers of apoptosis of PMNL in synovial fluid, as previously validated.[12] The strong association between pyknosis of PMNL and formation of CPM, their increasing number with time ex vivo, and the relation with ghost (dead) cell numbers, is all additional evidence that the pyknotic PMNL investigated were undergoing apoptosis and, under certain circumstances, were phagocytosed. Although such cells have previously been noted in synovial fluid[17 19] we believe this is the first time this has been investigated formally.

This cross sectional study of synovial fluid cytospins confirms that, as in vitro, apoptosis of PMNL appears to be essential for the formation of CPM (Reiter cells), though additional disease specific factors, possibly relating to macrophage function, seem to be crucial to the process.

We have shown (unpublished data) that PMNL stored in synovial fluid at 4°C under conditions that prevent phagocytosis or formation of CPM become entirely apoptotic, followed by cell lysis, over one to three days; counts of apoptotic PMNL in fresh synovial fluid rarely reach such levels, suggesting that macrophage phagocytosis of these effete PMNL, producing cells recognisable as CPM, may be an important mechanism limiting local tissue damage in inflammatory synovitis. Direct evidence for this mechanism has been obtained in short term fresh synovial fluid culture (table 10), where prevention of the formation of CPM by RPMI dilution was associated with decreased clearance of apoptotic PMNL. This is supported by our observations in the histological study that apoptotic PMNL and 'ghost' (autolysed) cell numbers are considerably lowered in synovial fluid in which CPM are formed.

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