A comparative quantitative morphometric study of cell apoptosis in synovial membranes in psoriatic, reactive and rheumatoid arthritis


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Abstract

Objectives. Inflammatory arthritides/synovitides such as psoriatic (PsA), reactive (ReA) and rheumatoid (RA) arthritis share numerous immunopathological features, but develop different patterns of joint involvement. To investigate whether distinctive cell apoptosis may play a role in this context, we have assessed synovial cell apoptosis in situ in PsA and ReA, and compared it with RA and 'non-inflammatory' controls.

Methods. TdT-mediated dUTP nick end-labelling (TUNEL) of DNA breaks complemented immunoperoxidase staining for CD68 or LCA as the specific cell markers.

Results. The proportion of apoptotic synovial lining cells was high in PsA, ReA and RA compared to values in controls (P < 0.05). No differences existed between these inflammatory arthritides in numbers or type of apoptotic lining cells. In RA, however, in contrast to PsA and ReA, apoptotic lining cells were clustered or, in a small subset of samples, were very low in number. Prominent apoptosis of inflammatory cells in the sublining in ReA has accounted for higher overall apoptotic cell numbers in synovial stroma (sublining + perivascular inflammatory cell infiltrates) in this condition than in RA or PsA (P < 0.05).

Conclusions. No disease-specific pattern in the phenotype of apoptotic synovial lining cells could be suggested in any of the inflammatory arthritides studied. However, topological differences in the lining and quantitative differences in the inflammatory cell apoptosis in synovial stroma may in part explain the occurrence of the prominent synovial lining cell hyperplasia distinguishing RA from ReA and PsA. On the other hand, relatively frequent inflammatory cell apoptosis may contribute both to the downregulation of synovial inflammation and to the control of synovial lining hyperplasia in ReA.

Key words: Psoriatic arthritis, Reactive arthritis, Rheumatoid arthritis, Synovial membrane, Apoptosis.

Apoptosis is a process characterized by a series of specific events leading to the activation of cellular nucleases, fragmentation of DNA and, eventually, to cell death. It differs from cell necrosis in that apoptotic cells are cleared from tissues without inducing a local inflammatory reaction. Apoptosis is not a 'random process', but is regulated both spatially and temporally by specific regulatory mechanisms, and by changes in the micromilieu of cells. In addition to local proliferation and cell recruitment from the circulation, cell turnover in tissues is directly dependent on the rate of cell apoptosis. Hence, apoptosis may govern the
morphofunctional characteristics of a given tissue. Adequate apoptosis is an important part of normal physiological processes such as tissue development, involution, maintenance of tissue homeostasis and immunoregulation. Disturbance in the apoptosis of specific cells has been implicated in the development of cancer, in persistence or dissemination of some viral infections, ischaemic injury, degenerative neural diseases, autoimmune conditions and rheumatoid arthritis (RA) [1–4].

It is assumed that, in RA, an imbalance between cell apoptosis and cell influx, along with cell proliferation in situ [5, 6], may account for synovial lining and inflammatory cell hyperplasia, the features believed to be relevant to the development of joint erosion or persistence of rheumatoid inflammation, or both [7, 8]. Indirect support for this hypothesis comes from the studies demonstrating apoptotic synovial cells and an eventual disturbance in some of the apoptosis-regulatory pathways in RA [8–16]. Nonetheless, the histo- and clinicopathological relevance of these disturbances in synovial cell apoptosis in RA is obscure, because it remains unknown which of them are ‘primary’ and which are merely ‘secondary’ (inflammation related). It is apparent in this context that comparative studies between synovial cell apoptosis and its regulation in various inflammatory arthritides, including RA, psoriatic (PsA) and reactive (ReA) [17–19], may be helpful in elucidating this issue.

Although synovial and immunocompetent cell responses in PsA and ReA share numerous immunopathological characteristics with RA [20–25], the clinical pattern of joint involvement in RA is distinct in that it is almost always characterized by erosion of hard tissues such as cartilage and subchondral bone. The destructive nature of RA may, to a large extent, depend on synovial tissue which undergoes ‘mesenchymoid transformations’ (e.g. aberrant expression of oncogenes, disturbance in the turnover of specific cells/cell proliferation) and, as a result, acquires destructive capacity against cartilage and bone. It is interesting, however, that such anticipations, based on RA studies, have not been supported by recent comparative studies of inflammatory arthritides. For example, these recent studies have revealed that in situ proliferation of synovial lining fibroblasts and synovial expression of numerous oncogenes, including those associated with cell turnover [25–30], are, in all established inflammatory arthritides, rather similar. This fact prompted us to extend these cross-sectional studies, and quantitatively and phenotypically to characterize synovial cell apoptosis in ReA and PsA, and compare it with that in RA and ‘non-inflammatory’ controls.

Materials and methods

Patients and samples

With the informed consent of 29 patients, synovial tissue samples (1–2 specimens from 27 knee, one hip and one elbow joints) were obtained at their open-joint surgery or diagnostic arthroscopy. These same synovial samples were used in a previous study [25]. Eight of the patients (two men and six women; mean age 47 yr, range 31–73) had RA [31] (mean duration of the arthritis 7.8 yr, range 3–15), eight (six men and two women; mean age 32.6 yr, range 7–43) had PsA [18] (mean duration of the arthritis 7.4 yr, range from 4 months to 25 yr) and seven (two men and five women; mean age 33.4 yr, range 19–53) had ReA (duration of the arthritis 3 days (two cases), 6 months (one case), 1 yr (one case), 2.5 yr (one case), 3 yr (one case), and in one case recurrent knee monoarthritis for 12 yr). Specimens from six patients (three men and three women; mean age 41.2 yr, range 28–63) served as ‘non-inflammatory’ controls; these synovial samples were from joints with osteoarthritis (OA) (n = 2) [32], meniscus lesion (n = 2), distortion of the knee joint (n = 1) and knee chondromatosis (n = 1).

All patients with PsA had psoriatic skin disease and were negative for rheumatoid factor. Of the patients with ReA, arthritis had been triggered by chlamydial infection in five cases, and in one by Salmonella infection, and in one case ReA had developed after pharyngitis (no Salmonella, Yersinia, Campylobacter or chlamydial antibodies were found in this patient’s blood). Synovial fluid cultures of ReA patients were negative. Five of the ReA patients were HLA B27+. All ReA patients recovered during the follow-up.

Of these 29 patients, none had previously undergone synovectomy. Of the RA patients, four had been receiving daily non-steroidal anti-inflammatory drugs (NSAID) alone, one chloroquine (Delagil®, 200 mg) + NSAID, one steroids (7.5 mg equivalent to prednisolone), one sulphasalazine (1.5 mg + steroids (10 mg equivalent to prednisolone) + NSAID; one of the RA patients received no systemic medication. Of PsA patients, one had been receiving sulphasalazine (1.5 g) + NSAID, one allopurinol + NSAID, two NSAID alone, and four received no systemic medication. Of the patients with ReA, two received no medication, five had been receiving NSAID; in one of the latter, antibacterial therapy had been initiated prior to synovial biopsy. Of the ‘non-inflammatory’ control patients, five had been receiving NSAID and one received no systemic medication.

Detection of apoptotic cells/TUNEL staining

Reagents: anti-Dig-AP (alkaline phosphatase-conjugated anti-digoxigenin antibody from Boehringer Mannheim, Indianapolis, IN, USA); BCIP (5-bromo-4-chloro-3-indolyl-phosphate from Sigma, St Louis, MO, USA); BSA (bovine serum albumin from Sigma); CHAPS (3-(3-chloramidopropyl) dimethylammonio-1-propanesulphonate from Sigma); dATP (2-deoxy-adenosine-5-triphosphate from Boehringer Mannheim); Dig-11-DUTP (digoxigenin-11-2-deoxy-uridine-5-triphosphate from Boehringer Mannheim, Germany); EDTA (ethylenediaminetetraacetic acid from Merck); NBT (4-nitroblue tetrazolium from Sigma); PFA (para-
formaldehyde from Fluka, Darmstadt, Germany); TdT + buffer (terminal deoxynucleotidyl transferase from Promega, Madison, WI, USA); Triton X-100 (t-octylphenoxypolyethoxyethanol from Sigma).

For detection of apoptotic cells, a slightly modified TdT-mediated dUTP-biotin nick end-labelling (TUNEL) method was applied, based on TdT-mediated labelling of 3'-OH DNA ends exposed in apoptotic cells [33, 34]. Briefly, 5-μm-thick serial sections from the formalin-fixed paraffin-embedded synovial samples were applied to APES (3-aminopropyl-triethoxysilane, from Sigma)-coated slides. Before staining, sections were heated at +60°C for 30 min, cooled, deparaffinized in xylene and dehydrated in absolute ethanol. Further, the sections were treated with a solution of absolute ethanol/acetic acid (2:3) for 20 min, with 5% H₂O₂ in methanol for 30 min, 94% methanol twice for 5 min, and 70% methanol for 5 min, and were left in 70% methanol overnight at +4°C. The sections were rinsed in graded methanol series (50% and 30%) and in PBT (phosphate-buffered saline containing 0.1% Triton X-100).

Cells in the tissue sections were permeabilized by application of a proteinase K 7 μg/ml (staining-to-noise ratio optimized by testing different proteinase K concentrations, such as 7, 14 and 21 μg/ml) solution in 50 mM Tris–HCl and 5 mM EDTA for 10 min at room temperature. The sections were rinsed twice for 5 min with PBT and post-fixed in fresh 4% PFA for 20 min. PFA was removed by two subsequent 5 min rinses in PBT.

TdT-mediated labelling of the fragmented DNA present in the apoptotic cells was carried out in a humidified box at +37°C by incubating tissue sections for 1 h in labelling mixture prepared freshly in the TdT buffer. The labelling mixture consisted of: (1) 1 nm Dig-11-dUTP; (2) 1 nm dATP; (3) 0.5% CHAPS; (4) 20 U TdT/75 μl of mixture. The labelling was terminated by transfer of the sections into 300 mM NaCl, 30 mM sodium citrate and 0.1% CHAPS for 15 min, and then by two subsequent rinses (for 20 min in total) with gentle rocking in TBT 0.1% CHAPS. Then, the sections were pre-blocked with 10% goat serum and 2% BSA in TBT overnight in a humidified box. Mouse embryo powder was made and alkaline phosphatase-conjugated anti-digoxigenin antibody was pre-absorbed as described elsewhere [33].

After the pre-blocking solution was drained off, pre-absorbed anti-digoxigenin antibody was applied onto sections and left overnight at +4°C in a humidified box.

Sections were rinsed with TBT (Tris-buffered saline, containing 0.1% Triton X-100) three times for 5 min, then three times for 30 min under continuous rocking. Thereafter, sections were rinsed for 5 min in alkaline phosphatase buffer (NTM), then rinsed twice for 5 min under continuous rocking in NTM containing 2 mM levamisole (to inhibit endogenous alkaline phosphatase). Staining was developed by applying to the sections a freshly prepared mixture of 33% rNBT (75 mg/ml solution in 70% dimethylformamide) and 175 μg/ml BCIP (50 mg/ml solution in absolute dimethylformamide) in NTM. The colour reaction was monitored under the microscope and terminated by extensive washing of tissue sections in PBT. Tissue sections were left for 4 h in PBT. Finally, the sections were rinsed extensively in distilled water, counterstained with Nuclear Fast Red (Fluka), briefly dried, and mounted with aqueous mounting media Glycergel (Dako, Glostrup, Denmark), or not counterstained, but processed by immunostaining (see below).

Double TUNEL/CD68 and TUNEL/LCA immunostaining

After TUNEL staining (see above), the sections were rinsed in distilled water three times for 5 min, following by a wash in 0.1 M phosphate-buffered saline (PBS), pH 7.4. Then the sections were serially incubated/treated with: (1) normal horse serum (diluted 1:50; Vector Laboratories, Burlingame, CA, USA) for 45 min; (2) primary antibody (for 1 h), a monocyte/macrophage marker mouse anti-human CD68 IgG (dilution 1:50, Dako) or mouse anti-human leucocyte common antigen (LCA; clones 2B11 and PD7/26, Dako, diluted 1:75), which reacts with epitopes on LCA chains with a molecular mass of 180, 190, 205, 220 kDa, labelling mainly lymphoid, but not non-haematopoietic cells (for references, see antibody specification) [this antibody is not designed as a CD45 and, in contrast to many other lymphoid and myeloid cell markers, is particularly recommended for use on routinely processed, paraffin-embedded specimens]; (3) secondary biotinylated horse anti-mouse IgG (diluted 1:130; Vector Laboratories) for 45 min; (4) avidin–biotin–peroxidase complex (diluted 1:100, Vector Laboratories) for 30 min; (5) 3,3’-diaminobenzidine tetrahydrochloride (DAB) (Sigma) in 0.006% H₂O₂ PBS solution. After being washed in tap water, the sections were rinsed in distilled water and mounted in aqueous mounting media Glycergel (Dako).

BSA, 0.1% in PBS, was used for dilution of serum and antibodies. Between each step (except after incubation with normal goat serum), the slides were rinsed three times for 5 min in PBS. Omission of the primary antibody (for procedure control), use of normal mouse IgG₁ instead of specific primary antibodies (for specificity of the staining) and exposure of tissue sections to DAB alone (for endogenous peroxidase) served as negative staining controls.

Microscopic evaluation and quantitative assessment of the synovial labelling

Microscopic examination and cell counting were carried out with the Leitz Diaplan lens system (Wetzlar, Germany). Morphological assessment of synovial lining hyperplasia and the extent of inflammation were by reference to the commonly utilized semiquantitative histological grading as follows. Synovial lining hyperplasia: no hyperplasia (1–2 cell layers thick), minimal hyperplasia (3–5 cell layers thick), moderate lining hyperplasia (>5 cell layers thick) and marked lining hyperplasia (>10 cell layers thick). Inflammatory mononuclear cell infiltration was graded as follows: no infiltrate, minimal infiltration, moderate infiltration (diffuse
and/or presence of aggregated perivascular infiltrates), marked infiltration (diffuse and/or presence of many aggregated perivascular infiltrates).

Assessment of synovial cell apoptosis (TUNEL staining) was performed separately (1) in the synovial lining, where the cells were counted through the whole thickness of the synovial lining, not the intimal monolayer only, and (2) in the synovial stroma comprising sublining areas and perivascular inflammatory cell infiltrates (in the inflammatory samples) up to the fibrocapsular tissue. In each of these two locations, 1000 consecutive cells were counted in the 3–4 consecutive synovial sections. The average percentage of the apoptotic lining and stromal cells was estimated separately for data presentation in each case. Synovial stromal cell apoptosis was assessed for its distribution to evaluate the presence of cell apoptosis separately in the sublining and in the inflammatory infiltrates as follows: 0, no cell apoptosis; +, occasional cell apoptosis; ++, scattered and/or focal cell apoptosis; ++++, extensive cell apoptosis.

Double TUNEL/CD68 and TUNEL/LCA staining results were expressed as the percentage of TUNEL+/CD68+ or TUNEL+/LCA+ cells in the total number of TUNEL+ cells, separately in synovial lining and stroma.

Each cell counting was performed by two observers, independently, one of whom was unaware of the patients’ identities or source of the sample.

Statistical analysis
Statistics were calculated with BMDP-PC 7.01 software. Medians plus interquartile ranges (Q3–Q1) were used for data presentation. The Siegel–Tukey test for scale difference served for comparison of the groups. Differences were considered significant at P ≤ 0.05.

Results

Standard histology
Of the eight RA cases, two revealed marked, four moderate, one minimal and one no synovial lining hyperplasia. In contrast, two of the seven ReA and five of the eight PsA cases showed no lining hyperplasia. Of the seven ReA, five, and two of the eight PsA cases showed minimal and only one PsA case moderate lining hyperplasia. Perivascular inflammatory cell infiltration was very similar in RA, ReA and PsA samples. Of the eight RA cases, two showed moderate, five marked and one minimal perivascular infiltration. Of the seven ReA cases, five showed moderate and two marked inflammatory cell infiltrates. Of the eight cases with PsA, one showed minimal, five moderate and two marked perivascular infiltration. (Case-referent description of these morphological features and the extent of the cell apoptosis is given in Fig. 5.)

Cellular pattern and synovial distribution of the TUNEL staining
TUNEL showed a mixed staining pattern, which was originally described as halo ring formation, chromosomal condensation, nuclear fragmentation or eosinophilic change in the cytoplasm [11], suggesting the presence of cells undergoing different stages of apoptosis.

TUNEL staining/apoptotic cells were observed in synovial lining and stromal (i.e. sublining and perivascular) locations (Figs 1–3). It should be noted, however, that in PsA apoptotic cells were preferentially situated in the lining (Fig. 2), whereas, as assessed by semiquantitative scoring of apoptotic cell distribution in the synovial stroma (see Table 1), in RA (Fig. 1)—and in ReA (Fig. 3) in particular—sublining areas adjacent to inflammatory cell infiltrates were also rich in apoptotic cells. In contrast to scattered apoptotic synovial lining cells in PsA and ReA (Figs 2 and 3), in five RA cases apoptotic cells were found clustered in limited areas of synovium (Fig. 1D); in fact, these RA cases were characterized by high numbers of apoptotic lining cells (see Fig. 4). No distinguishing features were observed in the distribution of apoptotic cells within the perivascular inflammatory cell infiltrates; similarly, in all types of arthritides studied, only relatively low numbers of apoptotic cells were scattered throughout the perivascular inflammatory cell-rich areas (Table 1, Figs 1–3).

Frequency of the synovial cell apoptosis in RA, PsA and ReA
Quantitative results of the TUNEL staining appear in Fig. 4. The percentages of apoptotic synovial lining cells in RA [median 2.1% (Q3–Q1) = 1.9%], PsA [median 1.4% (Q3–Q1) = 2.0%] and ReA [median 1.5% (Q3–Q1) = 1.0%] were significantly higher (P < 0.05) than in controls [median 0.1% (Q3–Q1) = 0.15%]. There were no statistically significant differences between RA, PsA and ReA in this respect (P > 0.05). Each of three RA cases, a subset with a notably lesser (and very similar) extent of apoptotic lining cells, showed moderate lining hyperplasia (Fig. 4), whereas another subset of RA samples, showing a notably greater extent of lining cell apoptosis, included cases with the differing degrees of lining hyperplasia (Fig. 4).

The percentages of apoptotic cells in the synovial stroma (sublining + inflammatory mononuclear cell-rich areas) in RA [median 2.0% (Q3–Q1) = 2.3%], PsA [median 1.1% (Q3–Q1) = 0.7%] and ReA [median 3.2% (Q3–Q1) = 2.4%] were significantly higher (P < 0.05) than in controls [median 0.6% (Q3–Q1) = 0.75%]. Control samples showed none or only occasional inflammatory cells, but the numbers of stromal cells were high enough for quantitative assessment. In addition, the percentage of apoptotic inflammatory cells was higher in ReA than in RA (P = 0.002) or PsA (P = 0.02), differences which were, apparently, accounted for by the differences in extent of sublining cell apoptosis in PsA, ReA and RA (see Table 1). The percentages of apoptotic inflammatory cells were similar in RA and PsA (P = 0.08).

Representative double-staining results are illustrated in Fig. 5. Of the apoptotic lining cells, on average 80% (range 70–100%) in RA, 86% (range 70–90%) in ReA
Synovial cell apoptosis in arthritis

Fig. 1. Distribution of apoptotic cells identified by TUNEL staining in rheumatoid arthritis (RA) synovium (original magnification ×160). (A) Fragment of synovial section demonstrating apoptotic lining (open arrows), sublining (arrows) and perivascular inflammatory cells (arrowheads) (a faint Nuclear Fast Red counterstain). (B) The same synovial area as in (A), counterstained with Nuclear Fast Red presented to demonstrate morphology. (C) Another case with RA showing minimal synovial apoptosis (a subset): apoptotic cells (arrows) are readily distinguished by the intense TUNEL staining (blue in original preparations) on the light Nuclear Fast Red counterstain. (D) Case with RA demonstrating clustered apoptotic cells (another subset) within a limited synovial area (long arrows); only a few apoptotic cells (short arrows) were found scattered in between such ‘apoptotic foci’ (light counterstaining with Nuclear Fast Red) (original magnification ×160).

and 84% (range 75–90%) in PsA were CD68+ cells. LCA+ apoptotic lining cells were mutually exclusive.

Of the apoptotic cells in the inflammatory mononuclear cell-rich areas, on average, 32% (range 10–60%) in RA, 38% (range 30–50%) in ReA and 33% (range 20–40%) in PsA were LCA+ cells. As estimated from the consecutive sections, of the apoptotic cells in the inflammatory mononuclear cell-rich areas, 70% (range 60–75%) in RA, 68% (range 60–80%) in ReA and 69% (range 60–80%) in PsA were CD68+ cells.

Discussion

Because of the possibly direct relationship between the development of synovial cell hyperplasia, joint-destructive capacity and chronicity of inflammation in RA, the mechanism of synovial cell turnover in this disease is an area of continuing investigation. In particular, recent studies have been focused on checking the hypothesis of impaired synovial cell apoptosis as a major contributor to synovial hyperplasia and, possibly, as a cause for the persistent ‘immunological inflammation’ in RA. In contrast, the relevance of synovial cell apoptosis to the pathogenesis of PsA and ReA, the other two inflammatory arthritides characterized by activation of the cellular immune response, has been less extensively addressed. This fact prompted us to assess synovial cell apoptosis in situ in PsA and ReA, and to compare it with RA and ‘non-inflammatory’ controls, an approach which has revealed several interesting observations. That is, we were unable to discover any disease-specific pattern in the phenotype of apoptotic synovial lining cells in PsA, ReA or RA. Notably, however, apart from local aggregates, the RA synovial lining and sublining contained only a few apoptotic cells, a finding which may, in part, explain the occurrence of the prominent synovial lining hyperplasia distinguishing RA from ReA and PsA.

As expected, all inflammatory arthritides showed a
Fig. 2. Distribution of apoptotic cells identified by TUNEL staining in reactive arthritis (ReA) synovium (original magnification ×160) (arrows as in Fig. 1). (A) Fragment of synovial section demonstrating apoptotic lining, sublining and perivascular inflammatory cells with a faint Nuclear Fast Red counterstain. (B) Same synovial area as in (A) counterstained with Nuclear Fast Red to demonstrate morphology. (C) Another case with ReA showing more intense infiltration with the inflammatory cells, but the same distribution of apoptotic cells with a light Nuclear Fast Red counterstain. (D) A negative staining control; in the labelling mixture, terminal deoxynucleotidyl transferase was replaced by buffer alone (no counterstaining). Note that, as in RA, in ReA many apoptotic cells appear in the sublining area.

Fig. 3. Distribution of apoptotic cells identified by TUNEL staining in psoriatic arthritis (PsA) synovium (original magnification ×160) (arrows as in Figs 1 and 2). (A) Fragment of synovial section demonstrating apoptotic of the lining and perivascular inflammatory cells. (B) The same synovial area as in (A) counterstained with Nuclear Fast Red to demonstrate morphology. Note that, in contrast to RA, in PsA relatively few apoptotic cells appear in the sublining areas.

frequency of apoptotic cells above that of our ‘non-inflammatory control’ samples. Considering, though, that some of the patients’ medications may have influenced the extent of their synovial cell apoptosis, we have performed a case-referent analysis (not shown), which revealed that the relatively high (or low) proportion of apoptotic synovial cells seen in some cases could not be explained by the treatment given to these patients. It should also be noted that quantitative similarities or differences observed in this respect between the patient...
Table 1. Distribution of apoptosis in synovial stroma

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<tr>
<th>Apoptosis in synovial stroma</th>
<th>In sublining</th>
<th>In perivascular infiltrates</th>
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<tr>
<td>No. of total cases of PsA</td>
<td>2/8</td>
<td>5/8</td>
</tr>
<tr>
<td>No. of total cases of ReA</td>
<td>3/7</td>
<td>4/7</td>
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<tr>
<td>No. of total cases of RA</td>
<td>4/8</td>
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0, no cell apoptosis; +, occasional cell apoptosis; ++, scattered and/or focal cell apoptosis; ++++, extensive cell apoptosis.

Fig. 4. Quantitative results of TUNEL staining in synovial membranes from rheumatoid arthritis (RA; n = 8), psoriatic arthritis (PsA; n = 8), reactive arthritis (ReA; n = 7) and Control (n = 6). Ln, synovial lining area; Str, synovial stroma (sublining + cell-rich inflammatory areas; in cases of Control = sublining + perivascular areas). Horizontal bars represent medians.

Fig. 5. Double TUNEL (nuclear, blue in original preparations) and immuno- (cytoplasmic, brown in original preparations) stainings used for phenotypic characterization of apoptotic cells (original magnification ×400). (A) (TUNEL/CD68) and (B) (TUNEL/LCA, clones 2B11 and PD7/26) represent the same synovial area in the consecutive synovial sections from RA. Single-TUNEL-labelled cells are indicated by thick arrows, double-TUNEL/immunostaining-labelled cells are indicated by thin arrows.
groups/arthritides should not be a result of sampling or measurement error, or both; we used the Siegel–Tukey test for scale difference, designed for comparison of groups with a wide scatter of individual values, to exclude such error at the probability level given [35].

Phenotypic characterization of cell apoptosis in synovium from different inflammatory arthritides revealed no differences in the type of apoptotic cells in the synovial lining in PsA, ReA or RA. Hence, although somewhat unexpectedly, our results appeared not to support the hypothesis that defective apoptosis of specific cells, e.g. synovial lining fibroblasts (CD68+), may be the feature distinguishing RA from other inflammatory arthritides. Any suggestion of a defect in the apoptosis-regulatory mechanisms in RA [8–16] seems to be reflected rather by the patchy involvement of synovial lining showing larger or smaller intervening areas with no (or few) CD68+ and CD68− apoptotic cells. Yet, although the overall figures for lining cell apoptosis were similar in all PsA, ReA and RA, the most prominent lining cell hyperplasia occurred in RA. This apparent discrepancy does not seem to be surprising, given the fact that, in RA, prominent synovial lining cell hyperplasia may be accounted for by both an over-exaggerated recruitment of circulating inflammatory cells/monocytes [36, 37] and the relatively (as compared with ReA) modest inflammatory cell apoptosis observed in synovial stroma/sublining. It should be noted, however, that this study assessed ‘a static change’ in synovial tissue which shows the presence of DNA fragments detected by the TUNEL assay. Apparently, we did not account for the timing of apoptosis or the dynamics of tissue clearance of ‘apoptotic debris’ [38]—the factors which can be speculated on as influencing any estimation of cell apoptosis in any given tissue.

Activation of the cellular immune response is central to the pathogenesis of all ReA, PsA and RA. In due course, however, only ReA is characterized by its normal downregulation and, therefore, by an inducible or spontaneous healing tendency of synovial inflammation/arthritides [39–42]. Our results here strongly indicate that this major difference between various inflammatory arthritides is also reflected by the relatively extensive cell apoptosis in the synovial sublining area in ReA, as compared with PsA or RA. Interestingly, aberrant regulation of lymphocyte perforin/granzyme-mediated apoptosis (impaired ability ‘to delete antigen presenting cells’ in synovium) has indeed been repeatedly advocated as one of the key mechanisms for disease chronicity in RA [43–51]. Still, in addition to the perforin/granzyme system, the cell microenvironment (e.g. synovial hypoxia in RA [52–54]), the Fas/FasL pathway [55] (e.g. its aberrant regulation is suggested in RA [15, 16]), other, as yet not well-established, factors [56–62], or their net effect, may appear to determine the extent of cell apoptosis in inflamed synovium. It would thus seem to be of major importance to establish which one of these apoptosis pathways has accounted for the eventual extent and topology of synovial cell apoptosis in the different arthritides demonstrated here.

Only a small proportion of apoptotic cells in perivascular inflammatory infiltrates were consistently identified in RA, PsA and ReA as lymphoid cells. These findings are in accord with the earlier reports which show, in RA, ReA, ankylosing spondylitis and OA, a relatively high expression of the apoptosis inhibitor bcl-2 in synovial tissue lymphocytes [29, 30]. It would seem, therefore, that similar expression of the adhesion molecules (e.g. ICAM-1 and VCAM-1) [37], and in situ lymphocyte proliferation [25], together with minimal lymphocyte apoptosis, are in accord with immunopathological similarities between PsA, ReA and RA. However, the pathogenetic role of even such low numbers of apoptotic lymphocytes in perivascular areas should not be underestimated merely because we lack information on their clonal characteristics in RA, PsA and ReA.

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