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Review

Synovial inflammation, immune cells and their cytokines in osteoarthritis: a review

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SUMMARY

Objective: Although osteoarthritis (OA) is considered a non-inflammatory condition, it is widely accepted that synovial inflammation is a feature of OA. However, the role of immune cells and their cytokines in OA is largely unknown. This narrative systematic review summarizes the knowledge of inflammatory properties, immune cells and their cytokines in synovial tissues (STs) of OA patients.
Design: Broad literature search in different databases was performed which resulted in 100 articles.
Results: Of 100 articles 33 solely investigated inflammation in OA ST with or without comparison with normal samples; the remaining primarily focussed on rheumatoid arthritis (RA) ST. Studies investigating different severity stages or cellular source of cytokines were sparse. OA ST displayed mild/moderate grade inflammation when investigated by means of haematoxylin and eosin (H&E) staining. Most frequently found cells types were macrophages, T cells and mast cells (MCs). Overall the number of cells was lower than in RA, although the number of MCs was as high as or sometimes even higher than in RA ST. Cytokines related to T cell or macrophage function were found in OA ST. Their expression was overall higher than in normal ST, but lower than in RA ST. Their cellular source remains largely unknown in OA ST.
Conclusion: Inflammation is common in OA ST and characterized by immune cell infiltration and cytokine secretion. This inflammation seems quantitatively and qualitatively different from inflammation in RA. Further research is needed to clarify the role of inflammation, immune cells and their cytokines in the pathogenesis of OA.

Introduction

Osteoarthritis (OA) is one of the most common rheumatic disorders. A majority of the elderly have radiographic or clinical evidence of OA[1]. For a long time, OA was considered a non-inflammatory condition and OA synovial tissue (ST) samples were used as controls for comparative purposes in studies that focused on rheumatoid arthritis (RA) pathogenesis. However, increasing evidence that inflammation is present in ST of OA patients[1–21] has brought to light the possibility that synovitis and the immune system could be active players in OA development and progression. Because immune cells and their cytokines may play an important role in the pathogenesis of OA and because a better understanding of the biological mechanisms involved in this process may lead to better therapies for OA patients, we performed a systematic narrative review to summarize the data published thus far regarding inflammation, immune cells and their cytokines in ST of OA patients.

Methods

In cooperation with a trained librarian, a broad search strategy in the following databases: Pubmed (1946–September 2011), Embase (Ovid-version 1980–September 2011) and Web of science (1945–September 2011) was composed (Fig. 1). Two different search strategies were used. The first consisted of the AND combination of following search terms: “osteoarthritis” and “synovium” as a major

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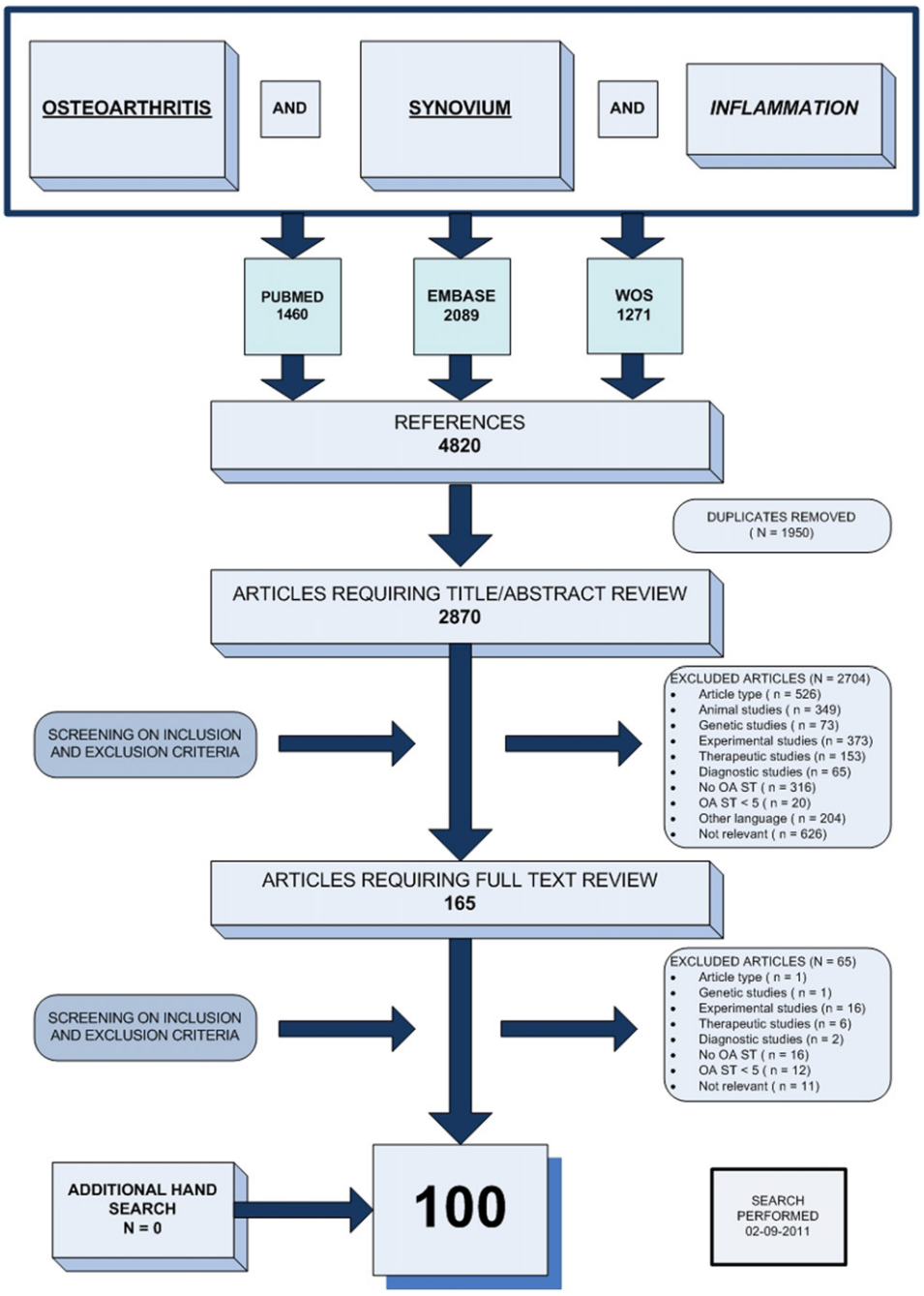

Fig. 1. Flowchart of first and second *(Italic)* search. WoS (Web of Science).

topic (major subject, title words). The second strategy consisted of the AND combination of three concepts; "osteoarthritis", "synovium" and "inflammation" (major or minor topic). All relevant keywords variations were used and search strategy was optimized for all consulted databases. See Appendix 1 for literature search details. Because the goal of this review was to summarize the existent knowledge about immune cells and their cytokines in OA ST, a broad search was initiated in which all studies were included that reported data regarding immune cells or their cytokines in OA ST, either in text or figures/tables. Moreover, studies that investigated at least five OA patients were included. 32 articles that investigated ST samples of less than five OA patients were excluded. Only studies investigating cytokines that are commonly associated with immune cells were included (e.g., interleukins (ILs)). Animal studies, experiments with non-immune cells and genetic studies were excluded. Studies concerning temporomandibular joints were excluded. After screening full text articles and after additional hand search of references and screening of related articles, a total of 100 articles remained.

Results

100 articles are included in this narrative review. 35 articles investigated OA ST by standard haematoxylin and eosin (H&E) staining (Table I). In 55 the focus was on different cell types (Table II), and in 37 articles the focus was on cytokines (Table III).

Only scarce data are available regarding the cellular source of cytokines in OA ST.

Table I
Studies that investigated ST in OA patients by means of H&E staining (phosphotungstic acid hematotoxylin (PTAH) and berlin blue)

Author	Arthropaties	OA phenotype	Methods	Results/conclusion OA ST
Arnoldi, 1980[23]	OA (24*), RA (12), N (8)	Hip arthroplasty	Grading by Salvati *et al.*, 1977	Inflammation moderate. Two histological types: proliferation and fibrous form
Da, 2007[24]	OA (41)	Knee needle arthroscopy	Synovial lining thickness, vascularity and infiltration	Mild to moderate inflammatory inflammation
Diaz-Torne, 2007[25]	OA (12), RA (10), N (9)	Knee biopsy or arthroplasty	Synovitis score Krenn *et al.*, 2002	Intermediate inflammation scores
Dolhain, 1996[26]	OA (8), RA (11)	Knee blind biopsy or arthroplasty	Inflammation score: SLL hyperplasia and infiltration	Inflammation score 3–8, RA 2–9 (score 0–15)
Fernandez-Madrid, 2005[11]	OA (9)	Knee arthroplasty	Histology	Mild chronic inflammation all cases
Fonseca, 2000[40]	OA (16), RA (26)	Arthroscopy	Grading: LL hyperplasia, vessel infiltrate + density, fibrosis, lymphocyte clusters + %	Mean global score (0–60) 11.1, RA 19.8
Furuzawa-Carballeda, 1999[19]	OA (5), RA (5), N (5)	Knee or hip arthroplasty	Histology: degree of inflammation	No difference between infiltrate type (perivascular and interstitial) or degree (varied moderate − abundant) between OA and RA
Gedikoglu, 1986[7]	OA (19)	Hip arthroplasty	Grading by Salvati *et al.*, 1977	All samples had features of inflammation: minimal inflammation 32%, moderate 53% and advanced 16%
Goldenberg, 1978[5]	OA (17)	Biopsies	Grading: hyperplasia, cellular infiltration and specific changes	Mostly quite normal in appearance, few moderate synovitis. 35% MNC infiltrate and 70% SLC hyperplasia
Goldenberg, 1982[6]	OA (15), N (4)	Knee or hip arthroplasty	Grading: hyperplasia, cellular infiltration and specific changes	87% samples abnormal; synovitis: 40% mild, 20% moderate, 27% markedly inflamed
Haynes, 2002[27]	OA (9)	Knee arthroplasty	Histology: inflammatory infiltrate	67% evidence inflammation, 44% large perivascular accumulations
Haywood, 2003[28]	OA (104)	Knee or hip arthroscopy (7) or arthroplasty (97)	Inflammation grading	7% no inflammation, mild inflammation 28%, moderate 35% and severe 31%
Houli, 1959[22]	OA (16)	Knee biopsies	Histopathological: cellular infiltration, fibrosis, etc	5/16 cellular infiltration, 11/16 fibrosis, oedema 11/16
Huss, 2010[29]	OA (13)	Arthroplasty	Histology: fibrin and aggregates	Dense fibrous bands, with aggregates with macrophages and lymphocytes
Johnell, 1985[30]	OA (16), RA (6)	Knee or hip arthroplasty	Histology: inflammatory cells	Inflammatory cells around small vessels and capillaries
Koizumi, 1999[31]	OA (22), RA (40)	Knee arthroplasty	Also PTAH, berlin blue staining. Scoring: proliferation synovial cells and stroma (infiltration, etc)	Score synovitis OA 91% <10 pts, all RA >11 pts (range 0—20 pts)
Korkusuz, 2005[13]	OA (10), N (6)	Hip or knee arthroplasty	Histology: hyperplasia and hypertrophy SLL, fibrosis	Hyperplasia and hypertrophy synovial LL suggesting an immunogenetic role
Krenn, 2006[32]	OA (212), RA (247), N (49)	Knee synovectomy	Synovitis score by Krenn *et al.*, 2002	Mean values synovitis score: control 1.0 (no synovitis), OA 2.0 (low-grade), RA 5.0 (high-grade). Significant differences between diagnoses and between high- and low-grade synovitis
Loeuille, 2005[3]	OA (39)	Knee arthroscopy	Grading: SLL cells, infiltration, surface fibrin, features vessels, fibrosis, perivascular oedema	Severe OA showed more fibrin, infiltrate and higher total score than mild OA
Loeuille, 2009[4]	OA (15)	Knee arthroscopy	Grading: SLL cells, infiltration, surface fibrin, features vessels, fibrosis, perivascular oedema	Increased number of synovial LCs, fibrosis, oedema and infiltration
Myers, 1990[2]	OA (29), N (14)	Knee arthroscopy	Synovitis: MNC infiltration graded 0–3	55% Moderate or severe synovitis. Normal ST 50% synovitis
Oehler, 2002[33]	OA (66), RA (22), N (8)	Arthroscopy (29) and arthroplasty (37)	Histology: degree infiltration (0–4) and hyperplasia	Four patterns: hyperplastic (72% arthroscopy group), lymphocytic infiltrates (28% arthroscopy, arthroplasty 38%), fibrotic (arthroplasty 32%), detritus rich (arthroplasty 24%)
Pearle, 2007[16]	OA (54)	Knee or hip arthroscopy or arthroplasty	Histology: grading synovial inflammation	50% Low-grade and 7% high-grade synovitis. No differences grade between arthroscopy and arthroplasty group
Pelletier, 1989[113]	OA (8)	Arthroplasty	Histology: morphological changes	Significant amount chroming inflammation, morphological changes were hyperplasia and hypertrophia SLC, MNC infiltrate and proliferation blood vessels
Pessler, 2008, second[34]	OA (25), RA (28), N (10)	Knee needle biopsy or arthroplasty	Synovitis score Krenn *et al.*, 2002, mean (SD)	Mean synovitis score 2.23 (low-grade), normal 1.38 (no synovitis) and RA 5.74 (high-grade)
Revell, 1988[9]	OA (38)	Knee or hip arthroscopy (3) or arthroplasty (35)	Histology: hyperplasia, lymphoid aggregates and fibrosis	Significantly more fibrosis and lymphoid aggregates than samples with machanical or traumatic background.

Table I (*continued*)

Author	Arthropaties	OA phenotype	Methods	Results/conclusion OA ST
Saito, 2002[35]	OA (19)	Knee arthroplasty	Histology: hyperplasia, infiltrate and vascular proliferation	All samples infiltrates and vascular proliferation. 45% SLL thickening
Sakkas, 1998[36]	OA (30), RA (10)	Knee or hip arthroplasty	Histology: hyperplasia, infiltrate	Histology: various degree of MNC infiltration, mild to moderate synovial hyperplasia. Inflammatory changes less prominent than in RA
Slansky, 2010[41]	OA (221) RA (341)	Knee synovectomy or arthroplasty	Synovitis score Krenn *et al.*, 2002	Median synovitis score +/− 2 (figure), RA 5.8 (figure), normal 0.6 (figure)
Smith, 1992[37]	OA (8), RA (16), N (5)	Knee arthroscopy or arthroplasty	Histology: hyperplasia and infiltrate LL and SLL	Variable LL hyperplasia as in RA. SLL: acellular till predominantly macrophage, lymphocyte or mixed infiltrates
Smith, 1997[18]	OA (40), N (23)	Knee arthroscopy (13) and arthroplasty (27)	Histology: hyperplasia, vascularity, cellularity and infiltrate	Low-grade synovitis, arthroplasty group highest degree of cellularity, vascularity and inflammatory infiltrate
Scanzello, 2011[42]	OA (20)	Arthroplasty and arthroscopy	Inflammation score: perivascular MNC infiltration (0–3)	25% No inflammation, 40% mild, 35% moderate and no severe inflammation
Soren, 1978[38]	OA (19)	Knee, hip, ankle synovectomy (8), arthroplasty (10), arthrodesis (1)	Histology: hyperplasia and infiltration	Some focal inflammatory infiltrates and moderate hyperplasia
Soren, 1988[43]	OA (74), RA (127)	Knee, hip arthrotomy	Histology: inflammatory features, hyperaemia, oedema, haemosiderin, fibrosis, other features	Presence synovial inflammation features in lesser incidence and intensity than RA
Thurkow, 1997[39]	OA (9), RA (10)	Knee needle biopsy	Histology: degree inflammation and infiltration	Inflammation 4.7 vs 11.4 RA (range 0–15), little infiltration

N, normal.
* Number of patients.

Only results relevant for OA are described. Thirty-three articles investigated exclusively OA ST, while the majority of the articles used OA samples for comparative purposes. Moreover, only a few studies compared samples of patients undergoing arthroscopy with patients undergoing arthroplasty in OA.

Synovitis in OA

Houli *et al.* were among the first to describe features of inflammation (31% samples showed cellular infiltration) in ST from OA patients[22]. Later, several studies confirmed the presence of inflammation in OA ST[2,5–7,9,13,16,18,19,23–43]. Histological features were hyperplasia, with an increased number of lining cells (LCs) and a mixed cellular infiltrate[2,6,7,9,18]. Overall, inflammation of OA ST was found to be less pronounced than in RA[19,23,25–27,30–34,36,37,39], but higher than in healthy controls[13,18,19,25,32–34,37,40,41,43,44].

Synovitis score

Different scoring systems for synovitis were used throughout the years, some designed specifically for OA[16], others with focus on RA[31,45]. An early scoring system of OA was designed by Salvati *et al.* for ST inflammatory responses in coxarthrosis[7,23]. In 2002 Krenn *et al.* developed a scoring system for the entire spectrum of rheumatic diseases including OA[46]. This system was based on the following morphologic alterations: hyperplasia/enlargement of synovial LCs, activation of resident cells/synovial stroma and inflammatory infiltration[46]. It is a validated scoring system that can accurately discriminate between high and low grade synovitis[32] and is frequently used[21,25,34,41,44].

With this scoring system a mild to moderate synovitis was found in OA (mean score around 2)[25,32,34,44,46] vs 1.4 for healthy controls and 5.7 for RA (scale 0–9). This mild to moderate synovitis was confirmed in other studies using author developed scoring systems[2,5–7,13,16,18,24,26,28,39]. Additional histological studies revealed that also fibrosis and detritus (non-living organic material) can be seen in OA ST[9,23,33,35].

Synovitis in different severity stages in OA

Tissues obtained from arthroscopy, reflecting early to established disease, were usually compared with arthroplasty, reflecting end-stage disease. Results vary, as one study reports a significantly increased mononuclear cell (MNC) infiltration in the arthroscopy samples[17], whereas others find these markers to be increased in the arthroplasty samples[2,3,18]. Pearle *et al.* did not find difference[16].

One study has extensively characterized the different inflammatory patterns and showed that hyperplasia and infiltrate are characteristic for the arthroscopy group, while fibrotic and detritus rich domains together with infiltrate are typical for arthroplasty group[33].

Cell types found in OA ST (Table II, Fig. 2)

Many studies investigated inflammatory cell types in OA ST (Table II)[8–12,17,20,21,24,25,27,28,30,33–37,44,45,47–77]. Several authors reported that macrophages and T cells are the most abundant cells[8,9,11,17,20,21,25,34,35,37,45,47,48,51,56,57,61,62,69,78]. Mast cells (MCs)[49,50,54,55,58–60,64,67,70,79], as well as B cells and plasma cells were found, although the last two in lower amounts than other cells[9,11,21,24,25,33–35,44,45,57,69,73]. Natural killer cells were detected in OA ST[65] and several authors describe the presence of low numbers of dendritic cells (DCs)[8,53,66,69]. Neutrophils were almost never found[21,34,35,44,48,62]. Overall OA ST contained fewer number of inflammatory cells than RA ST[25,30,36,37,44,45,56,57,63,65,68,69,71,75,78], but more than normal control ST[25,34,44,45,50,51,55,57,59,61–63,65,67,70,71,75,78].

One extensive study by Pessler *et al.* also investigated the relative abundance of immune cells in infiltrates and found that macrophages represented approximately 65%, T cells 22%, B cells 5% and plasma cells <1% of the infiltrate. MCs were not analysed in this study. Relatively more B cells were found in RA[44].

Macrophages

Macrophages were mostly distributed in the lining layer (LL)[20,25,28,33,37,44,48,56,61,62,78]. With increasing histological infiltration grade, both macrophage fraction areas as intensity of macrophage infiltration increased[17,28]. Subsets of macrophages, "classically activated"(M1) and "alternatively activated"(M2)[30,68], have not been investigated in OA ST. Although the importance of macrophages in OA ST is hypothesized[21,33,35,44,48], their role in human OA ST is still largely unknown.

Table II
Studies that investigated different cell types in ST in OA patients

Author	Arthropaties	OA phenotype	Methods	Results/conclusion OA ST
Andreu, 1991[47]	OA (5*), RA (7)	Arthroplasty	MNCs isolated. Pan-γ/δ T cell Mab TCR δ1	No γ/δ T cells expansion
Benito, 2005[17]	OA (25)	Knee arthroscopy (10) vs arthroplasty (15)	IHC Ab CD4, CD68	Significantly more T cells and Macrophages in arthroscopy group compared with arthroplasty group
Bondenson, 2006[48]	OA (19)	Knee or hip arthroplasty	Cell suspension ST. Flow cytometry analysis	Mainly fibroblast like synoviocytes. 2–7% macrophages, <0.5% neutrophils, <0.1% T Cells
Bridges, 1991[49]	OA (42), RA (48)	Knee, hip or wrist arthroplasty or synovectomy	Digest ST. Alcian blue staining. Histamine release assay	In digest 1.6% MCs, not significantly different from RA 1.3%. Histamine content comparable in OA and RA
Buckley, 1998[50]	OA (14), N (14)	Knee arthroplasty	IHC Ab chymase spec, tryptase. Double labelling procedure	Higher number MCs than control ST, distributed throughout ST. OA ST shift MC_{TC} to MC_T (77%) phenotype, normal ST MC_T (42%)
Cannons, 1998[51]	OA (8), RA (93), N (19)	Arthroplasty	T cells isolated assay hprt-mutant T cells, Mab CD3, CD8, TCR α/β, TCR γ/δ, CD29, flow cytometry	Isolated MNCs were predominantly CD4+ T cells. Number of hprt-mutant T cells lower than RA
Cauli, 1997[52]	OA (10), RA (10)	Unknown	IHC Ab cells: 27E10, CD14, 25F9, RM3/1. Ab cytokines: IL-1α, IL-1β, IL-1Ra Double staining for macrophages and cytokines	No difference subset macrophages between LL and SLL. Higher % mature macrophages, IL-1Ra+ than IL-1α+ in SLL. % 25F9+, IL-1+ α cells OA <RA. % 25F9+, IL-1Ra+ and RM3/1+, IL-1Ra+ similar OA and RA
Cauli, 2000[53]	OA (8), RA (8)	Arthroplasty	IHC Ab CD1a, CD1b, CD1c	Few CD1b and CD1c, no CD1a, similar to RA
Ceponis, 1998[79]	OA (12), RA 10)	Arthroplasty, arthroscopy	IHC c-kit and SCF. Toluidine blue staining	Increased densities c-kit+ synovial MCs. Mean number c-kit+ MC/mm^2 (135 ± 26) not significantly different from RA (85 ± 16)
Da, 2007[24]	OA (41)	Knee needle arthroscopy	IHC Ab CD3, CD20, CD138	No B lymph infiltration in 54%, mild in 32%, moderate/strong in 15% of the samples. Plasma cells 17%. No follicular DCs
Damsgaard, 1999[54]	OA (10)	Knee or hip arthroplasty	Giesma staining. Stereological microscopy MCs	Morphology: MCs found in vicinity blood vessels, not in LL. MCs 0.8% total number of cell profiles present in ST
Diaz-Torne, 2007[25]	OA (12), RA (10), N (9)	Knee biopsy or arthroplasty	ICH Ab CD3, CD68, CD20, CD38	Significantly more macrophages (LL and subintima (SI)) and T cells than gulf war veterans illness samples. B cells and plasma cells were sparse. Lower number inflammatory cells than in RA ST
Dean, 1993[55]	OA (36), RA (21), N (8)	Knee arthroscopy and arthroplasty	Toluidine blue staining and alcian blue safranin staining MCs	MCs most numerous in SI, number of deregulated MCs was greater in the superficial layer. MC numbers comparable to RA ST, higher than in control ST
Farahat, 1993[56]	OA (10), RA (10)	Arthroplasty	IHC Ab CD3, CD4, CD8, CD68, CD14, 27E10, RM3/1	Macrophages predominantly in LL, T cells mostly perivascular and in connective tissue layer not LL. Both T cells and macrophages in lower numbers than in RA
Fernandez-madrid[11]	OA (9)	Arthroplasty	IHC CD2, CD4, CD8, CD19, I3, RM3/1	MNC infiltrate composed of B and T cells, predominantly T cells (CD4 >CD8)
Fonseca, 2002[57]	OA (6), RA (6), N (3)	Arthroplasty	IHC and IF Ab CD68, CD163, CD14, CD19, CD45, CD4, CD3, CD8, double staining: IFN-γ	Macrophages found in I, SI and around lymphocytic clusters. T cells in SI. Fewer numbers of cells than RA. Some T cells stained for IFN-γ. In normal ST no cells stained for IFN-γ
Fritz, 1984[58]	OA (30), RA (90)	Unknown	Avidin-peroxidase vs toluidine blue and Giesma staining of MCs. Double staining method	Distribution MCs in SLL. Number of MCs significantly higher than in RA ST in sub synovial layer
Gotis-Graham, 1997[59]	OA (18), RA (16), N (15)	Arthroscopy or arthroplasty	IHC Ab chymase and tryptase	Number MCs higher in superficial layers than in deeper layers. Number of MCs significantly lower than in RA ST, higher than normal ST. Ratio MC_{TC}:MC_T 3:4, RA 3:2
Gruber, 1986[60]	OA (7), RA (8)	Unknown	Histamine release studies	Significantly lower histamine content than in RA ST
Haynes, 2002[27]	OA (9), RA (6)	Arthroplasty	IHC Ab CD4, CD8, CD28, CD69, CD40L	T cells major constituents of infiltrates. Aggregates: CD4+ >CD8+ cells. Some CD4+ cells were CD3−. Cellular activation marker (CD69) expressed by aggregated cells
Haywood, 2003[28]	OA (104)	Knee or hip arthroscopy (7) or arthroscopy (97)	IHC Ab CD14, CD31. Mean macrophage fractional area	Macrophages (LL) evident in inflamed ST samples. Mean macrophage fractional area 9.1%. Fractional area increases with increased inflammation
Helbig, 1988[61]	OA (25), RA (62)	Arthroplasty	Mab Ki-M6, Ki-M8, anti-HLA-DR, OKT 9	Mean HLA-DR+ similar RA. OKT9+ and ki-M8+ cells (macrophages) lower than in RA

Table II (*continued*)

Author	Arthropaties	OA phenotype	Methods	Results/conclusion OA ST
Hogg, 1985[62]	OA (8), RA (9)	Arthroplasty, arthroscopy	Mab 24, UCHM1, 44, 24, DA2, 5.5, 28	Less mature monocyte in intima, macrophages scattered intima, HLA-D-positive cells were synovial monocytes. Subset synovial cells was densly stained by monoclonal antibody 5.5 No neutrophils
Ishii, 2002[63]	OA (10), RA (10), N (10)	Arthroplasty	IHC Ab CD3, CD4, CD8	More CD3+ and CD4+ positive T cells than normal ST, but less than in RA ST. T cells found in SLL and DL, RA in all ST layers
Johnell, 1985[30]	OA (16), RA (6)	Knee or Hip arthroplasty	IHC Ab leu-1, 2a, 3a cells	Leu-1 T cells and Leu-3a, leu-2a sparse. Leu-3a near HLA-DR+ cells. T cells RA ST >OA ST
Kraan, 1999[45]	OA (17), RA (36), N (5)	Knee needle biopsy	IHC Ab CD3, CD4, CD8, CD68, CD22, CD38, CD55	Mostly T cells (CD4+ >CD8+) and macrophages, few B cells and plasma cells. Lower number of cells than RA ST, more than normal ST
Kopicky-Burd, 1988[64]	OA (22), RA (19)	Arthroplasty	Histology MCs, histamine content perchloric acid extraction	MCs in SI often in perivascular locations. Histamine content not significantly different (OA 5.4, RA 3.7)
Kummer, 1994[65]	OA (5), RA (5)	Knee Arthroplasty and arthroscopy	Mab granzymes A and B. IHC double staining UCHT1, MT310, DK25, CD3, CD4, CD8, CD16, CD56, CD15	2/5 samples OA granzyme A and B, 3/5 in RA, granzyme A and B positive cells mostly CD16+, CD56+ NK cells
Lebre, 2008[66]	OA (10), RA (20)	Arthroscopy	IHC Ab CD1c, CD304, CD3, CD8, CD11c, CD123	Both myeloid DCs and plasmacytoid DCs were observed in low numbers, mostly in SLL
Lindblad, 1987[8]	OA (10)	Knee arthroscopy	IHC Ab αLeu-1, αLeu-4, αLeu-2a, αLeu-3a, OKM1. Double staining with HLA-DR	All cell types, but mostly macrophages and T cells (predominantly T-helper phenotype) were found. HLA-DR+ cells near αLeu-3a
Lindblad, 1989[10]	OA (5)	Knee arthroscopy	IHC Ab αLeu-1, αLeu-4, αLeu-2a, αLeu-3a, OKM1. Double staining with HLA-DR	Local inflammation with T cells, B cells, plasma cells and HLA-DR-expressing DCs adjacent to CD4+ T-helper cells
Mitchell, 2008[78]	OA (10), RA (14), N (6)	Knee arthroplasty	IHC CD68+	Lower number macrophages than in RA ST, more than in normal ST
Nakamura, 1999[12]	OA (5)	Arthroplasty	IHC Ab CD3+	Mild or moderate infiltration of CD3+ T Cells observed in all samples in mainly perivascular areas
Nakano, 2007[67]	OA (12), RA (14), N (4)	Knee arthroplasty	Supernatants ST. Western blot and IHC Ab tryptase	MC tryptase activity similar in OA and RA, sign. higher than in control ST
Oehler, 2002[33]	OA (66), RA (22), N (8)	Arthroscopy (29) and arthroplasty (37)	ICH Ab CD3, CD4, CD8, CD20, CD68 and CD138	In ST with histological inflammatory features more B cells, T cells and plasma cells, compared with other histological patterns. More macrophages in lining LL than in RA ST or normal ST
Ogdie, 2010[21]	OA (31), RA (28), N (22)	Knee needle biopsy, arthroscopy and arthroplasty	IHC Ab CD3, CD15, CD38, CD20, CD68	Mostly T cells and macrophages, in LL. Little B cells and plasma cells and neutrophils. Intermediate cell densities in OA ST. CD68 distinguishes OA from RA and normal
Pawlowska, 2009[68]	OA (11), RA (11)	Knee synovectomy and hip arthroplasty	T cell population. FACS. Ab CD3, CD4. CD8, CD28	Lower number T cells than in RA ST, CD4+ >CD8+ cells. Relatively more CD28+ cells in older people, not sign
Pelletier, 1989[113]	OA (8)	Arthroplasty	Immunostaining studies. PAb human IL-1 (α and β)	Significant amount chronic inflammation. Significant amount IL-1 in MNC infiltrate SLL and at LL level
Pessler, 2008, first[44]	OA (25), RA (25), N (15)	Knee needle biopsies or arthroplasty	ICH Ab, CD3, CD20, CD38, CD68. Quantitative assessment (QA) and relative composition infiltrate (RC)	Mostly macrophages in LL and SI (RC ± 65%) and T cells in SI (RC ± 22% (40% CD8+)), no/few neutrophils and B cells and plasma cells in SI (RC ± 5% B cells, plasma cells <1%). RC similar normal ST, RA more CD20, CD38+, less CD68+
Pessler, 2008, second[34]	OA (25), RA (28), N (10)	Knee needle biopsies or arthroplasty	ICH Ab CD15, CD3, CD8, CD20, CD38, CD68. Quantitative assessments (QA)	QA: macrophages and T cells most common cells. Numbers lower than RA and higher than normal. Little B cells and plasma cells in OA ST
Pettit, 2001[69]	OA (10), RA (18),	Knee arthroplasty	IHC Ab C-19, (Rel-B), CD-20, CD3, CD68, HLA-DR	Perivascular MNC aggregates were small and few. Some had nRelB+ differentiated DCs, B cells, T cells and macrophages, 84% lacked nRelB+ diff DCs
Pu, 1998[70]	OA (13), RA (17), N (3)	Knee arthroplasty	Prussian blue staining MCs	MCs found near small blood vessels. MC count significantly higher than in RA ST and normal ST

(*continued on next page*)

Table II (*continued*)

Author	Arthropaties	OA phenotype	Methods	Results/conclusion OA ST
Revell, 1988[9]	OA (38)	Knee, hip arthroscopy (3), arthroplasty (35)	IHC Ab 24, 44, OKM1, 10.1, OKT4, OKT8, Pan B, 52, 29	Macrophages found in most samples at different locations, T cells 50% samples in SLL (OKT4+ >OKT8+), few pan B cells
Rollin, 2008[71]	OA (19), RA (11), N (3)	Knee arthroplasty	Flow cytometry CD5+, CD69+ IHC (OA $n = 9$, RA $n = 6$) Ab CD5, CD69	Tendency to higher expression lymphocytes than normal ST, lower than RA ST. Lymphocyte recent activation phenotype comparable RA ST and significantly higher than normal ST
Saito, 2002[35]	OA (19)	Knee arthroplasty	IHC Ab CD68, CD2, CD4, CD8, CD15, CD19, CD25, CD1a, toluidine blue for MC ($n = 11$)	Mostly T cells (CD4+> CD8+) and macrophages. 45% samples MCs in vicinity blood vessels. Macrophage/helper T cell interaction might be involved in synovitis in OA
Sakkas, 1998[36]	OA (30), RA (13)	Knee or hip arthroplasty	IHC Ab CD3, CD69, CD25, CD45RO, HLAII, CD38, CD43	65% samples lymphoid cells aggregates, containing predominantly CD3+ T cells. Expression of activation antigens, although in fewer numbers than in RA ST
Sakkas, 2004[72]	OA (19), RA (9), N (9)	Arthroplasty	Digested ST. Flow cytometry, IF, FACS and IHC. Ab anti-CD3ζ, anti-CD3ε	Decreased CD3ζ protein relative to CD3ε. Suggests chronic T cells stimulation and confirms T cell involvement in OA
Scanzello, 2009[20]	OA (11)	Knee arthroscopy (4) and arthroplasty (7)	IHC Ab CD3, CD8, CD68	Macrophages two distributions: in LL and scattered throughout SLL (similar in both groups) and lymphocytic accumulations (all arthroscopy samples, 57% arthroplasty samples)
Shiokawa, 2001[73]	OA (6)	Knee arthroplasty	Reverse transcriptase-PCR B-cell clonotypes DNA-cloning and sequencing	Infiltrating B-cells are oligoclonal (antigen-driven immune response may play a part in disease progress OA)
Smith, 1992[37]	OA (8), RA (16), N (5)	Knee arthroscopy or arthroplasty	IHC Ab CD3, CD8, CD4, IL-2r, CD25, CD5, CD14, CD64, CD11c	Mostly T cells (SLL) and macrophages (LL). IHC changes in similar as in RA ST, suggesting quantitative rather than qualitative differences
Steiner, 1999[74]	OA (5), RA (8)	Knee arthroplasty	IHC Ab CD3, CD4, CD8, CD20, CD45RA, CD45RO, CD68, IL-2, IL-4, IL-6, IFN-γ, TNF-α, double staining	80% samples only a few T cells, 20% had more T cells. CD4:CD8 ratio comparable to RA ST. No T cell cytokine expression in OA could be found
Warren, 1991[75]	OA (7), RA (8)	Arthroplasty or synovectomy	IHC CD3, CD4, CD8, CD45, CD45RA, CD45RO	Little lymphoid infiltrate
Weidler, 2004[76]	OA (8), RA (9)	Arthroplasty	mAB CD3, CD163, CD1a, CD1b, CD1c	Lower number of all investigated cells than in RA ST
Yamada, 2011[84]	OA (12), RA (18)	Arthroplasty	Isolation MNC, FACS IL-17A, CD57, CD45RO, CD28, IFN-γ, CD4, HLA-DR, CD69, CCR5 and intracellular staining	CD 4 T Cells expressed activation markers higher level than RA T cells. Th1 (IFN-γ) cells predominate in both OA as RA ST. Th17 (double producers IFN-γ and IL-17) were scarcely found
Yudoh, 2000[77]	OA (18), RA (25)	Arthroplasty or synovectomy	Lymphocytes isolated. Intracellular staining. FACS. Ab IL-4, IFN-γ, IL-2, IL-10, subtyping T cells by production cytokines	TH1 (IFN-γ, no IL-4) /TH2 (IL-4, no IFN-γ) ratio 1.5, 6.1 in RA. Tr1 (IL-10, no IL2 and no IL-4), thought to inhibit Th1-respons, higher than in RA ST. Other subsets CD4+ comparable RA

N, normal; diff, diffuse; IF, immunofluorescence; Ab, anti-bodies.
* Number of patients.

T cells

T cells were predominantly detected in the sublining layer (SLL) and to some extent in the deep layer (DL)[9,12,37,44,56,57,63]. T cells expressing activation antigens were found by different authors[27,36,72]. Sakkas *et al.* found that the MNC infiltrate consisted of T cells expressing early (cluster of differation (CD)69), intermediate (CD25 and CD38) and late (CD45RO human leukocyte antigen (HLA) class II) activation antigens in OA ST[36]. A later study by the same author showed a decrease in CD3ζ protein in OA ST, which is suggestive for a chronic T cell stimulation[72]. An altered ratio of CD4/CD8 T cells was found, showing a relative enrichment of CD4+ T cells[9,11,27,35,44,45,68,74]. Saito *et al.* reported a CD4+/CD8+ ratio in OA ST of 5:1, compared to normal ST, where the ratio is 2:1[35]. Steiner *et al.* found that the CD4+/CD8+ ratio in OA ST was comparable to RA ST[74]. Benito *et al.* reported that the abundance of CD4+ T cells was significantly greater in OA ST of the arthroscopy group compared to the arthroplasty group[17].

Several populations of T helper (Th) cells can be distinguished: Th1, Th2 and Th17 as well as regulatory cells (Th3 and Tr1) with unique function and unique cytokine patterns (Fig. 2)[80–83]. Different types of Th cells have been identified in OA ST, based on their cytokine profile upon *in vitro* activation. The Th1/Th2 cell ratio in OA reported by Yudoh *et al.* was 1.5 compared with 6.1 in RA[77]. Th1 cell was seen more frequently than Th17 cells[84]. One study by Yudoh *et al.* has investigated the presence of Tr1, a regulatory T cell that inhibits immune responses, in OA. The authors describe a higher abundance of Tr1 cells in OA ST compared to RA ST[77]. Moreover, several authors favour the concept that T cells play an important part in pathogenesis of OA[8,12,18,36,63,72,85].

MCs

Different authors detected MCs in OA ST[35,49,50,54,55,58–60,64,67,70]. Bridges *et al.* reported a percentage of 1.6 in OA ST[49]. Buckley *et al.*

Table III
Studies that investigated cytokines in ST in OA patients

Author	Arthropaties	OA phenotype	Methods	Results/conclusion OA ST
Alanärä, 2010[92]	OA (10*), RA (10)	Arthroplasty	Total RNA extraction, RT-PCR IL-10, IL-19, IL-20, IL-22, IL-24, IL-26	IL-10 and IL-26 expression similar as in RA, IL-19 lower than in RA ST. no IL-20 and IL-22 in OA
Brentano, 2009[98]	OA (8), RA (9)	Synovectomy or arthroplasty	IHC Ab IL-23 subsets p19 and p40. Total RNA extraction. RT-PCR IL-23p19 and IL-23p40	No/little IL-23 subsets. Subunits IL-23p19 and IL-23p40 not or weakly
Benito, 2005[17]	OA (25)	Knee arthroscopy (10) vs arthroplasty (15)	IHC Ab TNF-α, IL-1β	TNF-α and IL-1β expression significantly increased in arthroscopy group compared with arthroplasty group
Brenner, 2004[87]	OA (41)	Arthroscopy	RNA ST isolation, RT-PCR IL-6, IL-1β, IL-1α, TNF-α	No TNF-α or IL-1α. IL-1β in 14 of 16 investigated samples and IL-6 in all 17 samples
Cauli, 1997[52]	OA (10), RA (10)	Unknown	Ab cytokines: IL-1α, IL-1β, IL-1Ra Double staining for macrophages and cytokines	% 25F9+, IL-1Ra+ higher than IL-1α+ in SLL., IL-1+ α macrophages OA <RA. % 25F9+, IL-1Ra+ and RM3/1+, IL-1Ra+ similar OA and RA
Chu, 1991[99]	OA (8), RA (18), N (7)	Arthroscopy	IHC Ab TGF-β1	TGF-β1 detected, although in reduced quantities than in RA
Deleuran, 1992[107]	OA (8), RA (18)	Knee arthroplasty and arthroscopy	IHC IL-1α, IL-1R1, IL-1Ra	Fewer IL-1α and IL-1R1 in interstitium compare with RA. Similar percentage IL-1α in LL. Staining intensity IL-1α lower than in RA. IL-1Ra mostly LL as in RA
Deleuran, 1994[93]	OA (11), RA (13), N (6)	Arthroplasty	IHC and IF Ab IL-8	IL-8 LL and in deeper layers around vessels. Numbers significantly higher than normal ST, lower than RA ST (not significant)
Dolhain, 1996[26]	OA (8), RA (11)	Knee blind biopsy or arthroplasty	IHC Ab IFN-γ and IFN-γR	IFN-γ+ cells in 88% samples and IFN-γR in 38% samples, mainly around blood vessels and SL. Number cells lower than in RA
Doss, 2007[88]	OA (49)	Knee or hip arthroplasty	IHC Ab IL-6 counterstaining for different cells	Plenty IL-6 cells in synovial LL. Counterstaining revealed IL-6 production by plasma cells
Farahat, 1993[56]	OA (10), RA (10)	Arthroplasty	IHC Ab IL-1α, IL-1β, IL-6, TNF-α, GM-CSF	Expression IL-1α, IL-1β, IL-6, TNF-α, GM-CSF. Intensities expressions significantly lower than in RA ST. Differences in cytokine production OA and RA quantitative, not qualitative
Fonseca, 2002[57]	OA (6), RA (6), N (3)	Arthroplasty	IHC, IF Ab CD68, CD163, CD14, CD19, CD45, CD4, CD3, CD8, double staining: IFN-γ	Some T cells stained for IFN-γ. In normal ST no cells stained for IFN-γ
Furuzawa-Carballeda, 1999[19]	OA (5), RA (5), N (5)	Knee or hip arthroplasty	IHC Ab IL-8, IL-10. Total extracted RNA. RT-PCR IL-1β, TNF-α, IL-4, IL-6, IL-8, IL-10, IL-13, and TGF-β1	Little IL-8 and IL-10 expression, lower than RA ST, higher than normal ST. RT-PCR IL-1β, TNF-α, IL-6, IL-8, IL-10, and TGF-β1
Gracie, 1999[100]	OA (7), RA (18)	Arthroplasty	IHC Ab IL-18. Total RNA extraction OA (10), RA (20), RT-PCR IL-18	Little IL-18 compared to RA ST. IL-18 detected in OA ST by RT-PCR
Heinhuis, 2011[94]	OA (9), RA (15)	Arthroplasty	Total mRNA extraction. RT-PCR isoforms IL-32α, IL-32β, IL-32γ and IL-32δ	All isoforms detected IL-32 in OA. IL-32 γ significantly lower than in RA ST
Hulejova, 2007[89]	OA (55), N (10)	Hip arthroplasty	Tissue extracts OA ST. ELISA. Ab IL-1α, IL-10, IL-8, TNFα	IL-8 and IL-10 significantly higher than normal ST, TNF-α and IL-1α comparable to normal ST
Ishii, 2002[63]	OA (10), RA (10), N (10)	Arthroplasty	IHC Ab IL-4 and IFN-γ	IFN-γ+ five-fold higher than IL-4+ cells. Number both cells ± three-fold lower than in RA. Normal ST no IFN-γ and IL-4 staining
Jungel, 2004[106]	OA (6), RA (12)	Synovectomy or arthroplasty	IHC Ab IL-21R and IL-21 total RNA extraction. RT-PCR IL-21 and IL-21R	Weak IL-21R expression 33% samples. No IL21 in RA and OA ST. No IL-21 and IL-21R expression by RT-PCR
Kohno, 2008[95]	OA (10), RA (11)	Arthroplasty	cDNA samples RT-PCR IL-17	IL-17 gene not different from RA
Kragstrup, 2008[101]	OA (5), RA (8)	Arthroplasty	IHC Ab IL-20 and IL-24. Total RNA extraction, RT-PCR IL-20, IL-24 and TNF-α	Only few IL-20 positive cells. IL-24 staining present but weaker than in RA ST, with discreet staining of endothelial cells. IL-20 and IL-24 expression PRC same RA ST
Melchiorri, 1998[102]	OA (18), RA (6)	Knee arthroscopy	IHC Ab IL-1β, TNF-α	IL-1β and TNF-α expression mostly in LL and less in SLL. Expression both cytokines lower than in RA ST
Ning, 2011[90]	OA (23)	Knee replacement surgery (19), arthroscopy (4)	IHC Ab IL1β, TGF-β. Comparison K/L 2 or 3 vs K/L 4	IL-1β expression in both LL and SLL, higher expression in patients with K/L 2or 3 than in patients with K/L 4, TGF-β higher in K/L 4 than in patients with K/L 2 or 3
Saha, 1999[108]	OA (6), N (7)	Knee arthroplasty	*In situ* hybridization and IHC ICE	Expression IL-1β-converting enzyme (ICE) was detected in OA ST
Sakkas, 1998[85]	OA (16), RA (10)	Arthroplasty or synovectomy	IHC Ab IL-12p40, IL-12p70, counterstaining Ab CD68, CD20. RNA extraction. RT-PCR IL-12p40	31% samples IL-12p70+ cells (synovial LCs and monocyte/macrophages), no IL-12p40+. Presence IL-12p70 suggests an immunoregulatory role for IL-12. IL-12p40 transcripts detected, lower than in RA ST

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Table III (*continued*)

Author	Arthropaties	OA phenotype	Methods	Results/conclusion OA ST
Scanzello, 2009[20]	OA (11)	Knee arthroscopy (4) and arthroplasty (7)	IHC Ab rec-IL-15. Total RNA extraction. RT-PCR IL-1β, TNF-α, IL-2, IL-6 IL-15 and IL-21	Rec-IL-15 staining in LL and endothelium. IL-1β, TNF-α, IL-6 and IL-15 similar groups. IL-21 sign higher in arthroplasty group. IL-2 detectable over half patients
Shao, 2009[103]	OA (8), RA (12), N (7)	Unknown	Western blot IL-18, IL-18R, IL18BP. Total RNA extraction. RT-PCR IL-18, IL-18R and IL-18BP	IL-18 and IL-18R expression higher than control, lower than RA ST. Lower IL-18 and IL-18R than RA ST, higher N ST. Higher IL-18BP than RA ST, lower than N ST
Smith, 1997[18]	OA (40), N (13)	Knee arthroscopy (13) and arthroplasty (27)	IHC Ab IL-1α, IL-1β, IL-1Ra, TNF-α	In arthroplasty group higher production IL-1α, IL-1β and TNF-α than arthroscopy group. Similar IL-1Ra expression between OA groups and normal ST
Steiner, 1999[74]	OA (5), RA (8)	Knee arthroplasty	IHC: Ab IL-2, IL-4, IL-6, IFN-γ, double staining method	No T cell cytokine expression could be found
Suzuki, 2006[96]	OA (12), RA (21)	Arthroplasty	Total RNA extraction. RT-PCR TNF-α	TNF-α gene significantly higher in OA compared with RA
Szekanecz, 1995[104]	OA (10), RA (10), N (4)	Arthroplasty	IHC Ab TGFβ1, CD11c, f VIII H&E staining combined with IHC	Little TGF-β1 expression by LCs, macrophages and endothelial cells, less than RA ST, more than normal ST
Tanaka, 2001[105]	OA (12), RA (24)	Arthroplasty	IHC IL-18, western blot IL-18 (RA = 10, OA = 6) total RNA extraction. RT-PCR IL-18, IL-18R, IL-18Rβ	Small number IL-18 positive cells SLL lower than RA. All samples pro-IL-18, 67% no/weakly mature IL-18. IL-18 mRNA two of four samples. No IL-18R and IL-18Rβ.
Thurkow, 1997[39]	OA (9), RA (10)	Knee needle biopsy	IHC IL-15 Ab	Little IL-15 expression compared with RA
Wagner, 1997[97]	OA (5), RA (14)	Arthroplasty, synovectomy	Total RNA extraction, RT-PCR IL-1β, TNF-α, IL-2, IL-4, IL-5, IL-6 and IL-10	Expression IL-1β, TNF-α, IL-6 and IL-10 detected, expression IL-2, IL-4 and IL-5 not detected
Warren, 1991[75]	OA (7), RA (8)	Arthroplasty or synovectomy	IHC IL-2. Total RNA extraction, PRC IL-2	IHC: no IL-2 staining. mRNA IL-2 not increased above background
Wassilew, 2010[91]	OA (12)	Arthroplasty	RNA extraction, RT-PCR IL-1β, TNF-α	Both IL-1β and TNF-α found in ST. No differences between OA and traumatic joint disorder samples
Yamada, 2011[84]	OA (12), RA (18)	Arthroplasty	Isolation MNC, FACS IL-17A, CD57, CD45RO, CD28, IFN-γ, CD4, HLA-DR, CD69, CCR5 and intracellular staining	CD 4 T Cells expressed activation markers higher level than RA T cells. Th1 (IFN-γ) cells predominate in both OA as RA ST. Th17 (double producers IFN-γ and IL-17) were scarcely found
Yudoh, 2000[77]	OA (18), RA (25)	Arthroplasty or synovectomy	Lymphocytes isolated. Intracellular staining. FACS. Ab IL-4, IFN-\γ, IL-2, IL-10	TH1 (IFN-γ, no IL-4) /TH2 (IL-4, no IFN-γ) ratio 1.5, 6.1 in RA. Tr1 (IL-10, no IL2 and no IL-4) higher than in RA ST

N, normal; IF, immunofluorescence; Ab, anti-bodies.
* Number of patients.

found a percentage of 2.4 compared to 1.1% post-mortem and 1.3% in amputation controls[50]. Overall MC numbers were as high[49,50,55,79] or higher[58,70] compared to RA and higher than in controls[50,55,59,67,70,79], although one study reported lower numbers of MCs in OA compared to RA[59]. The highest abundance of MCs was found within the SLL[54,55,58,59] and around blood vessels[54]. The number of degranulated MCs was highest in superficial layers of OA ST, indicating their active state. In RA ST the number of MCs was highest in the capsule[55]. Despite a common origin, similar granulated morphology and functions, MCs are a heterogeneous group of cells. MCs can be subdivided into MCs containing only tryptase (MC_T) and in MC containing both tryptase and chymase (MC_{TC}) as defined by Irani *et al.*[86]. Buckley *et al.* investigated the MC subpopulations in OA ST vs control subjects. They found a striking shift in the relative proportions of MCs with a MC_{TC} phenotype to MCs with a MC_T phenotype. The number of MCs with a MC_T phenotype was higher in OA ST (median 53 MC_T/mm^2) than in post-mortem control ST (7.5 MC_T/mm^2) or amputation controls (12 MC_T/mm^2)[50]. Gotis-Graham *et al.* reported a lower ratio of MC_{TC}/MC_T in OA than in RA ST (3:4 vs 3:2)[59]. Similarly, a higher tryptase activity

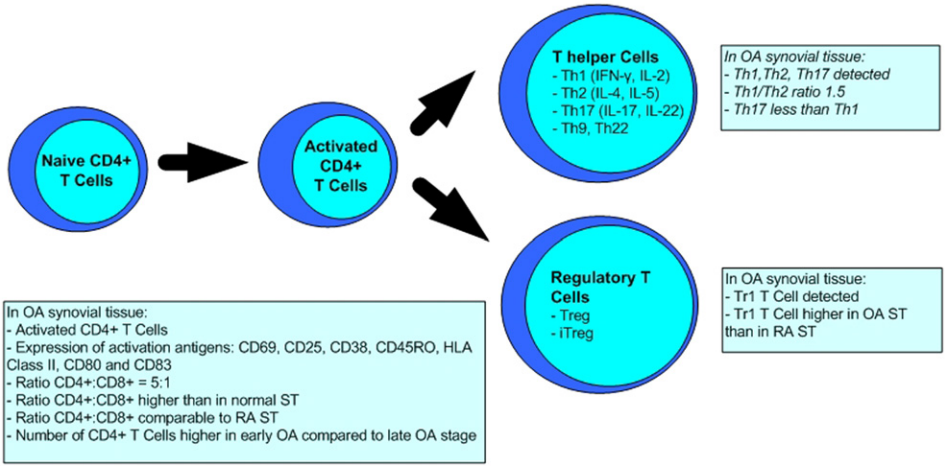

Fig. 2. T cells and T cell subsets in OA, with most important cytokines. Th cells (Th1, Th2, and Th17), T regulatory (Treg) cells and induced T regulatory (iTreg) cells (Th3 and Tr1).

of MCs in OA ST was found by Nakano *et al.*[67]. Furthermore the histamine content of MCs was the same or higher in OA ST than in RA ST[49] whereas a lower histamine release is reported[60,64].

B cells

Although present in low numbers, B cells and plasma cells were detected in OA ST[8,9,11,29,34,35,44]. However, their relative abundance is lower than in RA[44]. Da *et al.* reported that OA ST with increased inflammatory infiltrate contained relatively more B cells. Moreover, infiltrated B cells in ST of patients with OA were shown to be oligoclonal, suggesting an antigen-driven expansion[73]. Supporting this observation sequencing of the complementarity determing region (CDR) regions of these cells indicated that B cells have been clonally expanded. Therefore, a role for these cells during the course of OA cannot be excluded[24].

Other cells

Natural killer cells were not frequently studied but have been detected in OA ST[65]. Additionally very few DCs were seen in OA ST[8,53,66,69], although both plasmacytoid DC and myeloid DC subsets were found[66].

Cytokines associated with immune cells found in OA ST (Table III)

Although cytokines were extensively studied in animal and experimental studies, and in synovial fluid from OA patients, studies that primary focus on cytokines in OA ST are less abundant[17,18,20,87–91]. Various cytokines associated with immune cells have been detected in OA ST[17–20,26,39,52,56,57,63,75,85,88–105], and only a few studies reported no cytokine expression at all[74,106].

Cytokines detected in OA ST

IL-1β and tumour necrosis factor alpha (TNF-α) are both pro-inflammatory cytokines and are most frequently studied and detected[17–20,52,56,87,89–91,97,102,107]. These cytokines were most frequently seen in LL[52,102,107] and to a lesser amount in SLL[52,90,102]. Moreover, the presence of IL-1β-converting enzyme (ICE) indicates that IL-1β is not only present in OA ST, but that it can also be activated[108]. However, their cellular source is still largely unclear. Only one study used a double staining method for IL-1 and macrophage markers. In this study, macrophages were found positive for IL-1 and IL-1Ra.

Likewise, several other pro- and anti-inflammatory cytokines have been investigated in OA[19,20,26,39,56,57,63,74,75,77,85,87–90,92–95,97–101,103–106]. Among these, interferon gamma (IFN-γ), IL-2, IL-4, IL-6, IL-8, IL-18, transforming growth factor (TGF)-β and IL-10 have received most attention.

IFN-γ was detected by means of immunohistochemistry (IHC)[26,57,63]. The cellular origin of IFN-γ remains unclear. Although Yudoh *et al.* showed that ST T cells in OA can produce IFN-γ when stimulated *ex vivo*[77]. IHC studies using counterstaining for CD3 showed only very little[57] or no IFNγ-positive T cells *in situ*[74]. These data indicate that IFNγ-producing T cells might not be activated *in vivo* and that other cells could be the main source of this cytokine in OA.

IL-10 and IL-4 were also found in OA ST[19,63,77,89]. Yudoh *et al.* showed that ST T cells in OA can produce IL-10 and IL-4 when stimulated *ex vivo*[77]. Similarly to IFNγ, however, IHC using counterstaining for CD3 did not detect T cells positive for IL-4, indicating that T cells might not be the source of IL-4 in OA ST[74]. The cellular source of IL-10 has not been investigated yet.

Ex vivo stimulated T cells from OA ST were also found to produce IL-2[77], although this cytokine could not be detected by means of IHC in ST[74,97]. In conclusion, the cytokines secreted by ST T cells in OA are still unknown.

IL-6 has been detected in OA ST[19,56,88]. In the largest study by Doss *et al.*, IL-6 was detected in LL[88]. Stainings for various cellular markers indicated that plasma cells are the main source of IL-6[88].

Another cytokine associated with innate immunity, IL-8 was found to be produced in OA ST by means of IHC and enzyme linked immuno sorbent assay (ELISA) by several authors[19,89,93]. Deleuran *et al.* found IL-8 in OA samples predominantly in deeper layers and around vessels[93]. However, the cellular source of IL-8 remains unclear.

IL-18 and its precursor forms were also detected in several studies[100,103,105] and IL-18-producing cells were found predominantly in the SLL[105].

Finally, several studies found the presence of TGF-β in OA ST[90,99,104]. By means of H&E staining combined with IHC, it was found that macrophages were the primary immune cells expressing TGF-β[104].

For most of the cytokines described above, messenger Ribonucleic acid (mRNA) transcripts were also detected[19,20,75,85,87,91,92,94,96–98,100,101,103,105,106]. Additionally, some cytokines have only been investigated by means of mRNA analysis. Of those, IL-5, IL-13, IL-17, IL-19, IL-21, IL-26 and IL-32[19,20,92,94,95,97] could be detected, whereas IL-22 could not be detected in OA ST[92].

Differences in cytokine profiles between arthropaties

The overall conclusion emerging from the above-mentioned studies is that differences in cytokine expression between OA and RA are mainly quantitative, not qualitative[19,56]. In general, the number of cytokine-positive cells was lower in OA ST than in RA[19,26,39,56,63,74,89,93,98–105] and higher than in normal ST[18,19,51,57,63,93,103,104], indicating that OA is characterized by less inflammation than RA. Only one study found similar TNF-α and IL-1α expression in OA ST compared with normal ST[89].

Cytokine profiles in different severity stages in OA

Inflammation is believed to play a role in OA severity and progression. However, only very few studies exist that compare inflammation with different severity stages in OA. Moreover, only few cytokines were investigated in these studies. Moreover, conflicting findings exist in expression of IL-1 and TNF-α between ST of patients undergoing arthroscopy and patients undergoing arthroplasty. Benito *et al.* found a higher expression of both cytokines in the arthroscopy group, which is supported by results by Ning *et al.* The latter reported a higher IL-1β expression in patients with less severe disease (Kellgren–Lawrence score 2 and 3 vs 4). In the same study TGF-β expression was found to be lower in patients with less severe disease[90]. In contrast Smith *et al.* reported higher expression of IL-1α, IL-1β and TNF-α in the arthroplasty group[18], suggesting that additional studies are necessary for a firm conclusion.

Discussion

In this review we aimed at summarizing the published data regarding the presence and phenotype of immune cells and their cytokines in ST of OA patients. Our analyses revealed that synovitis is a common feature of OA and is usually characterized by the presence of infiltrating immune cells, such as macrophages, T cells and MCs. Likewise, among the cytokines investigated, the pro-inflammatory cytokines TNFα and IL-1β were most frequently detected in OA ST. Based on our analyses, we will discuss the limitations of our study, as well as possible research directions that could facilitate understanding the role of the immune system in OA.

Our search revealed that over 100 studies investigated and reported data on ST in relation with inflammatory markers. Because we intended to give a comprehensive overview of the literature, we included all studies in which more than five OA patients

participated, regardless of the disease they focus on. This low number of patients, however, implies that some of the presented data needs confirmation in additional studies or in larger cohorts. A meta-analysis of the studies was unfortunately not feasible, due to differences in methods and outcome measurements (e.g., different semi-quantitative systems and quantitative measures (IHC cells/mm^2 or cells/hpf or % infiltrate, % total ST)). Development of standardized methods for evaluating synovial inflammation could therefore be beneficial in the future.

Another limitation of the studies published thus far is that, while the knee is the most investigated joint (68% of articles that reported joint site), several studies exist that investigated both knee and hip but combined the data instead of presenting them separately. This complicates the interpretation of the results, as it is currently unclear how similar STs from different anatomical positions are in OA. It is likely that differences will be present, due to different influences from neighbouring organs, such as the infrapatellar fat pad (IFP) in the knee, which is lacking in the hip. We and others have previously shown that the IFP is a source of inflammatory mediators and could influence the pathophysiological processes in the knee joint[109–111]. Comparative studies of different ST would be of great interest for our understanding of the disease.

One of the most important conclusions of our review is that inflammation and synovitis are present in OA ST. More importantly, the features found by IHC seem to correlate with the inflammation observed by magnetic resonance imaging (MRI) Several histological features of ST (except oedema) and total composition score were significantly correlated (little-moderate) with MRI synovitis grade.

Much less is known, however, on the correlation between inflammation and clinical characteristics. In a study in 39 OA patients both function and pain were not associated with macroscopic and microscopic parameters of ST, visualized by H&E staining[3]. Because this is the only study that investigated correlation between clinical symptoms and ST, it remains largely unknown how features of OA ST translate to signs and symptom of OA in patients. Addressing this question in future studies will likely constitute a considerable step forwards to a better understanding and potentially treatment of OA patients.

The role of the immune system and of cytokines in OA is still poorly understood. Although several studies have shown the presence of different immune cells in ST in patients with OA, only few have attempted to further characterize these cells phenotypically and functionally. For example, it is still unclear which cytokines are secreted by macrophages, T cells and MCs, the most abundant immune cell populations in OA ST. Moreover, it is unclear how their phenotype relates to clinical symptoms or radiological features.

Pain is one of the most important features of clinical OA. Although the biological mechanisms involved in pain are still largely unclear, it has been suggested that local inflammation could play an important role[16]. This hypothesis is also supported by the finding that TNF-α concentration in synovial fluid was found to correlate with pain in knee OA patients[112].

In conclusion, more detailed knowledge of the immune cells and their cytokines in synovitis is important for a better understanding of clinical features in OA, including pain.

Comparison between OA and other arthritides or between different clinical phases of OA (early and late) could also offer insight into the role of synovial inflammation in disease progression. Several studies summarized in this analysis have addressed this topic. Unfortunately, the data are contradictory probably due to different definitions used for “early” or “late” OA; some authors found synovitis and cytokine expression more pronounced in patients with “late” OA undergoing arthroplasty[2,3,18], others declared the opposite[17,90] or did not find difference[16]. Longitudinal studies in different OA populations might offer a more definite conclusion.

Table IV
Research agenda for future investigations of ST in OA patients

Research agenda
• To investigate severity and histological features at different OA severity stages
• To correlate histological synovial inflammation severity and histological features with clinical parameters (pain/function)
• To investigate course/persistence of synovial inflammation during OA disease course in longitudinal studies
• To investigate the role of synovial inflammation in disease progression in longitudinal studies
• To further investigate cellular source of cytokines in ST of (different stages) OA patients
• To further investigate different subtypes of cells in ST of (different stages) OA patients
• To investigate presence of cells and cytokines in relation to disease severity and clinical features (pain/function) in (different stages) OA patients

Studies comparing OA with RA point largely to the same direction; the number of infiltrating immune cells as well as the expression of cytokines were higher in RA than in OA ST and both were higher than in normal ST. This is in line with the clinical observations that OA is less inflammatory than RA. However, there is one population of immune cells that is enriched in OA compared to RA, namely MCs. This indicates that the inflammation in OA may be less than in RA, but could be qualitatively different. This intriguing finding was confirmed by several studies and point to MCs as potentially important players in synovitis in OA. Future studies are needed to elucidate the role of MCs in OA.

In conclusion, our study indicates that inflammation is commonly detectable in OA ST and is characterized by immune cell infiltration and cytokine secretion. This inflammation seems in some aspects qualitatively different from the inflammation in RA. Future studies are needed to elucidate the role of different immune cells types and their cytokines in the development and progression of OA and their association with the different clinical features of the disease. Based on our results we suggest the research agenda depicted in Table IV.

Author contributions

Following authors participated in design of the study: de Lange-Brokaar BJE, Ioan-Facsinay A, van Osch GJVM, Zuurmond A-M, Schoones J, Toes REM, Huizinga TWJ, Kloppenburg M. Interpretation of data was done by following authors: de Lange-Brokaar BJE, Ioan-Facsinay A, Kloppenburg M. Drafting of manuscript was done by de Lange-Brokaar BJE, Ioan-Facsinay A, van Osch GJVM, Zuurmond A-M, Schoones J, Toes REM, Huizinga TWJ, Kloppenburg M. Final approval of manuscript was provided by following authors: de Lange-Brokaar BJE, Ioan-Facsinay A, van Osch GJVM, Zuurmond A-M, Schoones J, Toes REM, Huizinga TWJ, Kloppenburg M.

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Conflict of interests
None.

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Appendix 1. Literature search details

Database	Strategies	Number of references	Number of unique references
PubMed	1. ("osteoarthritis"[Majr] OR osteoarthritis[ti] OR osteoarthritic[ti] OR Osteoarthritides[ti] OR Osteoarthrosis[ti] OR Osteoarthroses[ti] OR "Degenerative Arthritides"[ti] OR "Degenerative Arthritis"[ti] OR (oa[ti] AND knee) OR arthrosis[ti] OR **"degenerative joint disease"[ti] OR "degenerative joint diseases"[ti]**) AND ("Synovial Membrane"[Majr:noexp] OR synovium[ti] OR "Synovial Membrane"[ti] OR "Synovial Membranes"[ti] OR "Synovial tissue"[ti] OR "synovial tissues"[ti] OR Synovialis[ti]) 2. ("osteoarthritis"[Majr] OR osteoarthritis[tiab] OR osteoarthritic[tiab] OR Osteoarthritides[tiab] OR Osteoarthrosis[tiab] OR Osteoarthroses[tiab] OR "Degenerative Arthritides"[tiab] OR "Degenerative Arthritis"[tiab] OR (oa[tiab] AND knee) OR arthrosis[tiab]) AND ("Synovial Membrane"[Majr:noexp] OR synovium[tiab] OR "Synovial Membrane"[tiab] OR "Synovial Membranes"[tiab] OR "Synovial tissue"[tiab] OR "synovial tissues"[tiab] OR Synovialis[tiab]) AND (inflammatory[tw] OR "inflammation"[mesh] OR inflammation[tw] OR "Synovitis"[mesh] OR synovitis[tw])	1460	1460
EMBASE (OVID-version	1. (exp *osteoarthritis/OR (osteoarthritis OR osteoarthritic OR Osteoarthritides OR Osteoarthrosis OR Osteoarthroses OR "Degenerative Arthritides" OR "Degenerative Arthritis" OR arthrosis OR "degenerative joint disease" OR "degenerative joint diseases").ti OR (oa.ti AND knee.mp)) AND (*synovium/ OR ("Synovial Membrane" OR synovium OR "Synovial Membrane" OR "Synovial Membranes" OR "Synovial tissue" OR "synovial tissues" OR Synovialis).ti) 2. (exp *osteoarthritis/OR (osteoarthritis OR osteoarthritic OR Osteoarthritides OR Osteoarthrosis OR Osteoarthroses OR "Degenerative Arthritides" OR "Degenerative Arthritis" OR arthrosis OR "degenerative joint disease" OR "degenerative joint diseases").ti,ab OR (oa.ti AND knee.ti,ab)) AND (*synovium/ OR ("Synovial Membrane" OR synovium OR "Synovial Membrane" OR "Synovial Membranes" OR "Synovial tissue" OR "synovial tissues" OR Synovialis).ti,ab) AND (exp *inflammation/ OR inflammation.ti OR inflammatory.ti OR exp *Synovitis/ OR synovitis.ti)	2.089	1018
Web of Science	1. TI=((osteoarthriti* OR Osteoarthritides OR Osteoarthrosis OR Osteoarthroses OR "Degenerative Arthritides" OR "Degenerative Arthritis" OR arthrosis OR (oa AND knee)) AND (synovium OR "Synovial Membrane" OR synovium OR "Synovial Membrane" OR "Synovial Membranes" OR "Synovial tissue" OR "synovial tissues" OR Synovialis)) 2. TS=((osteoarthriti* OR Osteoarthritides OR Osteoarthrosis OR Osteoarthroses OR "Degenerative Arthritides" OR "Degenerative Arthritis" OR arthrosis OR (oa AND knee)) AND (synovium OR "Synovial Membrane" OR synovium OR "Synovial Membrane" OR "Synovial Membranes" OR "Synovial tissue" OR "synovial tissues" OR Synovialis) AND (inflammat* OR synovitis))	1.271	382
Total		4820	2.860

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