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Is There an Association Between Synovial CD30+ T Lymphocyte Count and Chondral Lesion Depth?☆

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ABSTRACT

Background: Exact role of the inflammation in osteoarthritis is still unclear, but it is thought to originate from synovitis due to micro-crystals or breakdown products of the cartilage.
Objective: To determine the effect of CD30+ T lymphocytes on the development of osteoarthritis by comparing the lesion depth and synovial CD30+ count in patients with chondral lesions undergoing knee joint arthroscopy.
Design: A total of 79 patients with chondral lesions detected during arthroscopy were categorized in 4 different groups based on chondral lesion classification. CD30+ lymphocyte counts were calculated using flow cytometry on synovial fluid samples obtained at the time of initial entrance into the joint and compared between the groups. In addition, biopsy samples obtained from the suprapatellar bursa were stained for histologic examination to identify existence of CD30+ lymphocytes in the synovium.
Results: Although there were no significant differences between the first 3 groups in terms of synovial fluid CD30+ lymphocyte counts, patients in Group IV had significantly higher counts (6.2 8 [2.48] vs 2.51 [1.84], 2.97 [2.40], and 3.80 [2.07], respectively; $P < 0.05$). Except for a single patient with a Grade III chondral lesion, there were no cases of CD30 positivity in synovial tissue. Also there was a correlation between CD30 levels and chondral lesion depth when controlled for age.
Conclusions: Our results indicate higher CD30+ lymphocyte counts in patients with modified Outerbridge Grade IV chondral lesions than in other groups. The origin of the CD30+ lymphocytes may not be the synovial tissue per se. Thus, it was hypothesized that the injured chondral tissues and the associated subchondral structures might have been the source of CD30+ lymphocytes with a possible influence on the development of osteoarthritis.

Introduction

Osteoarthritis (OA) is not only the most common form of joint disease but also a major cause of disability. Knee is the most frequently involved joint. The etiology is not yet fully understood.[1,2] OA is a heterogeneous disease.[3] Rheumatologists generally consider OA to be a noninflammatory disease,[3] although patients with OA often exhibit inflammatory infiltrates in the synovial membrane. These infiltrates mostly consist of T cells and macrophages.[4–10] Recently Skapenko et al[11] reported that T cells and cytokines are not only present in inflammatory diseases, but also significantly contribute to the perpetuation of chronic inflammation such as OA. The exact role of the inflammation in OA is still unclear, but it is thought to originate from synovitis due to microcrystals or breakdown products of the cartilage.[3]

T lymphocytes represent the single most important cell type for immune functions and are responsible for specific immunity, which is regulated by nonantibody-dependent cells involved in the process. T cell populations are not homogenous and consist of different subgroups, each with a specific function and structure. In addition to common surface antigens found in all T lymphocytes (such as CD2, CD3, and CD5), other surface molecules also exist that are used to discriminate different T lymphocyte subgroups.[4–12] The CD30 gene is located on chromosome 1 at 1p36. It appears to be a lymphoid activation gene and is part of the nerve growth factor/tumor necrosis factor superfamily. The protein product is a 120 kDa transmembrane glycoprotein. Its ligand, CD30L, has homology to tumor necrosis factor. The transmembrane glycoprotein is often referred to as the true CD30 antigen.[13] CD30 is a costimulatory molecule that plays an important role in the generation of T cell responses and regulation of the balance between Th1- and Th2-type immune responses.[14] Reactive inflammatory disorders may contain

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Table I
CD30 counts in the groups adjusted for age.

	Group I (n = 19)	Group II (n = 15)	Group III (n = 22)	Group IV (n = 23)	*F*	*P*
	Mean % (SD)					
CD30	2.51 (1.84)	2.97 (2.40)	3.80 (2.07)	6.28 (2.48)	12.3	0.001

a significant number of CD30+ cells mimicking lymphoproliferative disorders clinically or histologically.[15]

Our study examines for the first time the role of CD30+ T lymphocytes in the pathogenesis of OA among 79 patients with cartilage damage. CD30+ T lymphocyte count was determined in joint fluid samples from 79 patients undergoing knee arthroscopy; presence of CD30+ T lymphocytes in synovial tissues was explored and the association between CD30+ T lymphocyte count and the severity of arthroscopic chondral lesions was evaluated.

Methods

Seventy-nine patients (38 men and 41 women) attending outpatient clinics due to OA and meniscopathy were included in this study. Patients with rheumatologic conditions were excluded. Arthroscopy was performed in 37 right and 42 left knees. During the creation of an access point for arthroscopy, a 1.5-cc joint fluid sample was obtained in test tubes containing EDTA. Subsequently 2 μL DakoCytomation (Carpinteria, CA) monoclonal antibody CD30 Clone Ber-H2 was added into the tube and the percentage of CD30+ T lymphocytes was determined through cell count of labeled lymphocytes using an Epics XL-MCL device (Beckman Coulter Inc, Brea, CA). Then, during arthroscopy, synovial biopsy samples were obtained from the suprapatellar bursa and stained immunohistochemically for the presence of CD30+ T lymphocytes. We used anaplastic large cell lymphoma tissue as a positive control for CD30 immunohistochemistry. Chondral lesions were examined during arthroscopy and were graded using the modified Outerbridge classification scheme.[16] Patients were categorized in 4 different groups as follows: Group I, no chondral lesions or Grade I lesions according to the modified Outerbridge grading system; Group II, patients with modified Outerbridge Grade II lesions; Group III, patients with modified Outerbridge Grade III lesions; and Group IV, patients with modified Outerbridge Grade IV lesions.

Statistical analyses

Data are presented as mean (SD). Kolmogorov-Smirnov test was used to test the distribution of data and 1-way ANOVA was applied for determining the between-group differences. A *P* value < 0.05 was considered significant. The group responsible for the difference was selected using Scheffe test and we used ANCOVA for multivariate correlation.

Results

Seventy-nine patients (38 men and 41 women) attending outpatient clinics due to OA and meniscopathy were included in this study. Mean (SD) age was 39.2 (7.6) years (range, 26–75 years). Patients' chondral lesions detected during arthroscopy were categorized into 4 different groups based on chondral lesion depth as determined by the modified Outerbridge classification scheme. Nineteen Group I, 15 Group II, 22 Group III, and 23 Group IV patients were evaluated. CD30+ lymphocyte counts were calculated using flow cytometry on synovial fluid samples obtained at the time of initial entrance into the joint and compared between the groups.

In addition, biopsy samples obtained from the suprapatellar bursa were stained for histologic examination to identify existence of CD30+ lymphocytes in the synovial tissue.

Significantly higher CD30+ T lymphocyte counts were found in patients with Grade IV chondral lesions (6.28 [2.48]) compared with those with Grade I, II, or III lesions (2.51 [1.84]; 2.97 [2.40]; or 3.80 [2.07], respectively) ($P < 0.05$) (**Table I**).

Histologic presence of CD30+ T lymphocytes could be demonstrated with immunohistochemical staining of the synovial tissues in only 1 patient with Grade III lesions. No other patients had CD30+ cells detected immunohistochemically (see **Table II** and the **Figure**). There were significant differences in terms of CD30 levels among the 4 groups. Additionally, there were significant differences in terms of age among the groups. In multivariate analyses, CD30 levels were correlated with adjusted-for-age and modified Outerbridge stages ($P < 0.05$) (**Table I**).

Discussion

To our knowledge, ours is the first study to examine the role of CD30+ T lymphocytes in the pathogenesis of OA. For this purpose CD30+ T lymphocyte count was determined in joint fluid samples from patients undergoing knee arthroscopy, presence of CD30+ T lymphocytes in synovial tissues was explored with biopsy, and the association between CD30+ T lymphocyte count and the severity of arthroscopic chondral lesions was evaluated. Patients with Grade IV chondral lesions had significantly higher CD30+ T lymphocyte counts compared with the other 3 groups. Except for a single specimen, histologic examination did not reveal any CD30+ T lymphocytes in synovial tissues, supporting strong evidence for the argument that the origin of the CD30+ T lymphocytes was not the synovial tissue. Thus, we believe that CD30+ T lymphocytes in the knee joint probably originate from the damaged chondral tissue and the adjacent subchondral tissue.

CD30+ anaplastic large cell cutaneous lymphomas show a better prognosis. Thus, CD30 positivity is a very important prognostic factor for T-cell lymphomas of the skin, with CD30 negativity being associated with a much more aggressive clinical course and poor prognosis.[17]

Table II
Histologic and immunohistochemical findings.

Group	n	Biopsy finding		Synovial fluid lymphocytes flow-cytometry		CD30 immunohistochemistry
		Normal	Chronic synovitis	CD30+	CD30–	+ Staining
I	19	4	15	1	18	0
II	15	3	12	3	12	0
III	22	3	19	4	18	1
IV	23	4	19	6	17	0
Total	79	14	65	14	65	1
Ï/2 and *P* value	0.45/0.92					

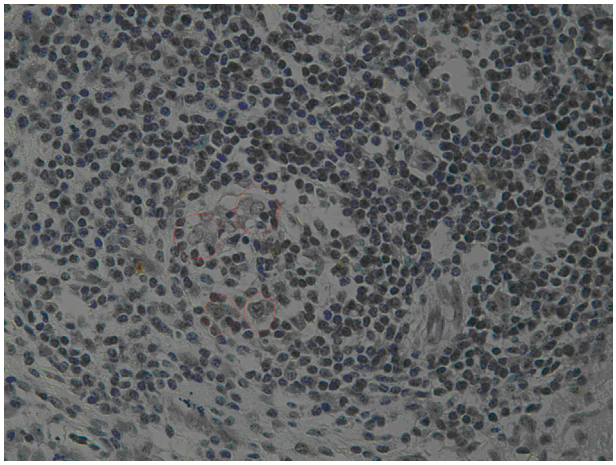

Figure. Biopsy sample showing CD30+ T lymphocyte positivity (red circles) in Group III.

In a study by Castilo et al[18] the role of interleukin-2, soluble interleukin-2 receptor, interleukin-10, interferon-γ, tumor necrosis factor-α, and CD30 was examined in patients with hepatitis B virus infection. These investigators found higher levels of interferon-γ and tumor necrosis factor-α during the early stages of the disease and lower levels during the healing period. On the other hand, despite higher CD30 levels at early stages, they tend to decrease with the appearance of the antibody to the hepatitis B surface antigen and normalization of liver enzymes.

Okumura et al[19] found high CD30 levels in patients with certain autoimmune conditions of the thyroid gland; for example, Hashimoto thyroiditis and Grave's disease. Patients experiencing a thyrotoxic episode had higher CD30 levels with a sharp decrease after the thyrotoxic attack. The same authors also regarded CD30 as a reliable marker for the disease activity for autoimmune conditions.

In light of the abovementioned data, CD30+ T lymphocytes appear to play a counter-regulatory role in inflammatory conditions due to their anti-inflammatory effect, ability to regulate the growth and differentiation of T lymphocytes, and ability to increase the release of anti-inflammatory cytokines from Th2 lymphocytes via CD30 stimulation. Our hypothesis was that there would be a correlation with CD30 levels and lesion severity in patients with knee OA.

Most of the histopathologic studies of OA have focused on the joint cartilage and bone tissue and there is a relative scarcity of data on synovial reactions, immunopathology, and the role of T lymphocytes in OA. Focal lymphocyte infiltration also occurs in patients with OA, although not as marked as in patients with rheumatoid arthritis.

Upper layers of the synovium have been shown to harbor CD4+ T and CD8+ T lymphocytes in patients with OA or rheumatoid arthritis, respectively. The synovial inflammation observed in most patients with OA is considered a secondary process that starts with the release of macromolecules from cartilage breakdown. Inflammation of the synovial membrane causes increased synthesis of cytokines, which in turn causes further breakdown of the cartilage and inflammation.[20]

Damage to chondral tissue is related to the inflammation of synovial tissue. In our study, a majority of study patients had signs of chronic synovitis in their synovial biopsy samples. A parallel increase in the severity of synovitis is expected with increased injury in the cartilage. However all groups in our study had marked synovitis without a significant difference. Particularly in Group IV, where the subchondral tissues are exposed, the high CD30+ T lymphocyte count could be explained by an immunologic effort to suppress synovial inflammation.

It has been reported in the literature that the prevalance and the degree of OA increases with age.[21,22] The authors investigated inflammatory factors associated with OA in aged cohort and anti-inflammatory response to inflammatory stress may protect against OA.[23] In our study we suggest that increased age may contribute to depth of chondral lesions and also increased CD30+ T lymphocyte levels. In addition, CD30 count independently predicted depth of chondral lesions in our study.

Deeper chondral damage was associated with higher CD30+ T lymphocyte count in synovial fluid.

Conclusions

It appears that CD30+ T lymphocytes may play a role in the pathogenesis of OA, CD30+ T lymphocytes in synovial tissue originate from the damaged cartilage and subchondral tissues, and CD30+ T lymphocytes may play a counter-regulatory role in the inflammatory process in the synovium due to their anti-inflammatory effects.

Acknowledgments

Drs. Guney, Karaman, and Oner conceived and designed the experiments. Dr. Kafadar performed the experiments. Dr. Deniz analysed the ımmunohistochemistry.

Conflicts of Interest

The authors have indicated that they have no conflicts of interest regarding the content of this article.

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