Decreased Expression of the CD3ζ Chain in T Cells Infiltrating the Synovial Membrane of Patients with Osteoarthritis

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Received 24 April 2003/Returned for modification 16 July 2003/Accepted 2 October 2003

Osteoarthritis (OA) is a heterogeneous disease which rheumatologists consider to be noninflammatory. However, recent studies suggest that, at least in certain patients, OA is an inflammatory disease and that patients often exhibit inflammatory infiltrates in the synovial membranes (SMs) of macrophages and activated T cells expressing proinflammatory cytokines. We report here that the expression of CD3ζ is significantly decreased in T cells infiltrating the SMs of patients with OA. The CD3ζ chain is involved in the T-cell signal transduction cascade, which is initiated by the engagement of the T-cell antigen receptor and which culminates in T-cell activation. Double immunofluorescence of single-cell suspensions derived from the SMs from nine patients with OA revealed significantly increased proportions of CD3ζ-positive (CD3ζ+) cells compared with the proportions of CD3ζ-positive (CD3ζ+) T cells (means ± standard errors of the means, 80.48% ± 3.92% and 69.02% ± 6.51%, respectively; P = 0.0096), whereas there were no differences in the proportions of these cells in peripheral blood mononuclear cells (PBMCs) from healthy donors (94.73% ± 1.39% and 93.79% ± 1.48%, respectively; not significant). The CD3ζ+ cell/CD3ζ− cell ratio was also significantly decreased for T cells from the SMs of patients with OA compared with that for T cells from the PBMCs of healthy donors (0.84 ± 0.17 and 0.99 ± 0.01, respectively; P = 0.0302). The proportions of CD3ζ+ CD3ζ+ cells were lower in the SMs of patients with OA than in the PBMCs of healthy donors (65.04% ± 6.7% and 90.81% ± 1.99%, respectively; P = 0.0047). Substantial proportions (about 15%) of CD3ζ+ CD3ζ− negative (CD3ζ−) and CD3ζ− negative (CD3ζ−) CD3ζ− cells were found in the SMs of patients with OA. Amplification of the CD3ζ and CD3ζ transcripts from the SMs of patients with OA by reverse transcriptase PCR consistently exhibited stronger bands for CD3ζ cDNA than for CD3ζ cDNA. The CD3ζ/CD3ζ transcript ratio in the SMs of patients with OA was significantly lower than that in PBMCs from healthy controls (P < 0.0001). These results were confirmed by competitive MIMIC PCR. Immunoreactivities for the CD3ζ protein were detected in the SMs of 10 of 19 patients with OA, and they were of various intensities, whereas SMs from all patients were CD3ζ+ (P = 0.0023). The decreased expression of the CD3ζ transcript and protein in T cells from the SMs of patients with OA relative to that of the CD3ζ transcript is suggestive of chronic T-cell stimulation and supports the concept of T-cell involvement in OA.

Osteoarthritis (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 (SM) (16, 19, 26, 37, 48, 51, 60). These infiltrates mostly consist of T cells and macrophages (16, 19, 26, 37, 48, 51, 60). The infiltrating T cells, at least in advanced OA, have many of the features found in rheumatoid arthritis (RA), including the following: (i) they often exhibit a nodular pattern (51, 60); (ii) they express early, intermediate, and late cell surface activation antigens (51); (iii) they express a TH1 cytokine pattern (18, 51, 52, 60); and (iv) they contain substantial proportions of oligoclonal T cells [S. R. Scanzello, L. I. Sakkas, N. Johanson, and C. D. Platsoucas, Arthritis Rheum. 42(Suppl.):S257, 1999 (abstract); S. R. Scanzello, L. I. Sakkas, N. Johanson, and C. D. Platsoucas, Scand. J. Immunol. 54(Suppl. 1):59, 2001 (abstract)], suggesting an antigen-driven immune response. Antigen-driven activation of T cells is thought to be important in the pathogenesis of RA (15, 45, 50).

T cells recognize peptides bound to self major histocompatibility complex class I or class II through their alpha/beta (αβ) T-cell antigen receptor (TCR). In addition, other αβ TCR-positive (TCR+) T cells that recognize nonpeptide antigens have been identified (56, 72). The engagement of the TCR with antigen initiates a signal transduction cascade which culminates in T-cell activation. The CD3ζ chain plays a very important role in this pathway. Signal transduction involves a series of tyrosine phosphorylations and activation of protein tyrosine kinases (68). One of the earliest events upon TCR engagement with antigen is the phosphorylation of the CD3ζ chain, which leads to activation of the ZAP-70 protein tyrosine kinase (6, 68). ZAP-70 protein tyrosine kinase activates the phospholipase Cγ1, which in turn increases intracellular Ca2+ and activates protein kinase C (49, 68).

Defective signal transduction with diminished tyrosine phosphorylation of the CD3ζ chain was found in synovial fluid (SF) T cells from patients with RA, and this was associated with decreased CD3ζ chain protein expression (32). Decreased...
CD3ε protein expression was also reported in peripheral blood T cells from patients with RA (31) and systemic lupus erythematosus (SLE) (27) and in tumor-infiltrating lymphocytes (TILs) from patients with renal (12), colorectal (36, 38), and ovarian [23; J. Pappas, A. D. Wolfson, C. W. Helm, D. P. Barton, A. D. Tsygankov, and C. D. Platsoucas, FASEB J. 10:A1472, 1996 (abstract); J. Pappas, A. D. Wolfson, W. Jung, C. W. Helm, A. D. Tsygankov, and C. D. Platsoucas, J. Allergy Clin. Immunol. 99:S447, 1997 (abstract)] carcinoma and Hodgkin’s disease (47). In the study described in this report, we investigated CD3ε chain expression in the SMs from patients with OA at the transcript and protein levels.

MATERIALS AND METHODS

Patients. Nineteen patients with OA (35) 9 females, 10 males; mean age, 57 years; age range, 36 to 70 years) and nine patients with RA (7 females, 2 males; mean age, 64.8 years; age range, 51 to 74 years) were included in this study. Patients with OA had primary OA (17 patients) or secondary OA due to avascular necrosis (2 patients). All patients with OA were treated with nonsteroidal anti-inflammatory drugs, whereas patients with RA were treated with nonsteroidal anti-inflammatory drugs and intramuscular gold (three patients) or methotrexate (one patient). Nine healthy controls (two females, seven males; mean age, 33.9 years; age range, 25 to 49 years) were also included in the study. SM specimens were obtained at surgery during joint replacement. The use of these specimens has been approved by the Institutional Review Board of Temple University Hospital.

Preparation of single-cell suspensions from SMs. SM tissues were finely minced with scissors and digested at 37°C for 2 h in RPMI 1640 culture medium supplemented with 5% fetal calf serum, garamycin (10 µg/ml), collagenase I (1.5 mg/ml; Sigma), collagenase IV (0.25 mg/ml; Sigma), and DNase I (0.15 mg/ml; Sigma). The digests were sequentially passed through a 200-µm-pore-size sieve (Fisher Scientific) and a 70-µm-pore-size sieve (Becton Dickinson). Single-cell suspensions were collected by centrifugation on a Ficoll-Hypaque density cushion. Peripheral blood mononuclear cells (PBMCs) from healthy donors were used for methodology control in this study and were prepared by centrifugation on a Ficoll-Hypaque density cushion. These cells were immediately used for immunofluorescence staining.

Antibodies. A phycoerythrin (PE)-conjugated anti-CD3ε monoclonal antibody (MAb; mouse immunoglobulin G1 [IgG1]; clone UCHT1; PharMingen, San Diego, Calif.) and an anti-CD3ε MAb (mouse IgG1; clone 2H2D9[Ti-2]; Coulter/Immunotech) were used for flow cytometry. The anti-CD3ε MAb (clone UCHT1; Novocastra, Newcastle upon Tyne, United Kingdom) and anti-CD3βε (Coulter) were used for immunohistochemistry.

Immunofluorescence and flow cytometry. Cells (10⁶/ml) were at first fixed in 4% paraformaldehyde in phosphate-buffered saline (PBS) at room temperature for 10 min and perméabilized by incubation at room temperature for 7 min in 0.1% saponín-0.2% bovine serum albumin-PBS solution. Double labeling for CD3ε-positive (CD3ε⁺) and CD3ε-negative (CD3ε⁻) cells was carried out by standard protocols. Briefly, the cells were incubated with anti-CD3ε MAb and then with fluorescein isothiocyanate (FITC)-conjugated anti-mouse IgG (indirect immunofluorescence). Next, the cells were incubated with PE-conjugated anti-CD3βε MAb (direct immunofluorescence). The cells were incubated in a total volume of 100 µl on ice and were washed at 4°C in 0.1% saponín-0.2% bovine serum albumin-PBS. Negative controls included cells incubated alone without MAb or IgG antibodies and cells incubated with FITC-conjugated anti-mouse IgG alone (omission of primary antibodies). Fluorescence-activated cell sorter analysis, after gating for lymphocytes only, was carried out within 3 h of staining.

Immunohistochemistry. Six-micrometer-thick cryostat SM sections were air dried for 1 h and fixed in 4% paraformaldehyde in PBS for 30 min. Endogenous peroxidase activity was blocked by incubation with 0.3% H₂O₂ in ice-cold methanol, and the cells were perméabilized by incubation in 0.1% saponín-2% fetal calf serum-PBS. Then the sections were stained for CD3ε by the avidin-biotin indirect immunoperoxidase method as described previously (51), with the exception that incubations and washings were carried out in 0.1% saponin-PBS. Staining for CD3ε was carried out as described previously (46).

RT-PCR. SM tissue samples were homogenized in tissue grinders (Kontes), and total RNA was obtained by using RNeasy A B (Tel-Test). First-strand cDNA was synthesized from 5 µg of RNA by using Superscript II reverse transcriptase (RT) and 0.5 µg of oligo(dT) as a primer (GibcoBRL) and was kept at −30°C until it was used. CD3βε and CD3ε DNA was detected by 35-cycle PCR, with each cycle consisting of the following incubation steps: 94°C for 45 s, 61°C for 45 s, and 72°C for 90 s, with a final extension at 72°C for 7 min, as described previously (51). The primers (5’ to 3’) used for CD3ε amplification were CTCGACTGAGCATCATCTC and GATCCTGTCGATCC (3’ end), and those used for CD3βε amplification were ACAGAGCCTTGGCTTGACATTC (5’ end) and TAGGGTTCCCTGGGCTGCT (3’ end) (Bio-Synthesis) (17). The primer sequences span at least one intron, so a PCR product of a larger size would be obtained if any contaminating DNA was present. In addition, β-actin was also amplified as a control (51). After gel electrophoresis the PCR products were visualized under UV light, and the densities of the bands were measured by densitometry with Sigma gel software. In some experiments the PCR products were also quantitated by a competitive MIMIC PCR (53).

Quantitative PCR. CD3ε and CD3βε transcripts from SM specimens from five patients with OA were quantitated by competitive MIMIC PCR, as described previously (38, 51, 53). Internal nonhomologous competitive fragments (MIMIC DNA) for each transcript were constructed according to the instructions of the supplier (Clontech, Palo Alto, Calif.). Serial PCR mixtures containing a constant amount of sample cDNA (50 ng of RNA equivalents) were spiked with decreasing concentrations of MIMIC DNA. The PCR products were separated by electrophoresis through an ethidium bromide-stained 1.6% agarose gel and quantitated by comparison of the intensities of the transcript and the MIMIC bands. When the intensities of the target DNA and the MIMIC DNA product bands were equal, the amount of target DNA was equimolar to the amount of MIMIC DNA prior to amplification.

RESULTS

Double immunofluorescence staining with an anti-CD3ε MAb and an anti-CD3βε MAb, followed by flow cytometry analysis of single-cell suspensions derived from the SMs from TABLE 1. Proportions of CD3ε⁺ and CD3ε⁻ cells from the SMs of patients with OA and peripheral blood of healthy individuals (controls) analyzed by double immunofluorescence and flow cytometry

| Study subject | % CD3ε⁺ cells | % CD3ε⁻ cells | CD3ε⁺ cell/CD3ε⁻ cell ratio |
|---------------|---------------|---------------|---------------------------|
| Patients      |               |               |                           |
|               | A            | B            | C                         |
| 1             | 92.5         | 78.7         | 0.851                     |
| 2             | 87.2         | 84.3         | 0.967                     |
| 3             | 79.1         | 69.2         | 0.785                     |
| 4             | 94.1         | 85.4         | 0.907                     |
| 5             | 74.9         | 53.3         | 0.712                     |
| 6             | 69.1         | 60.1         | 0.870                     |
| 7             | 91.2         | 86.4         | 0.947                     |
| 8             | 59.6         | 70.0         | 0.853                     |
| 9             | 76.6         | 76.8         | 1.003                     |
| Mean ± SEM    | 80.48 ± 3.92 | 69.02 ± 6.51 | 0.84 ± 0.06               |
| Controls      |               |               |                           |
|               | A            | B            | C                         |
| 1             | 93.4         | 90.5         | 0.969                     |
| 2             | 93.8         | 90.3         | 0.963                     |
| 3             | 91.0         | 89.7         | 0.986                     |
| 4             | 98.5         | 95.1         | 0.965                     |
| 5             | 97.7         | 96.3         | 0.986                     |
| 6             | 86.2         | 91.8         | 1.000                     |
| 7             | 97.6         | 97.0         | 0.994                     |
| 8             | 99.1         | 98.4         | 0.993                     |
| 9             | 95.3         | 95.0         | 0.997                     |
| Mean ± SEM    | 94.73 ± 1.39 | 93.79 ± 1.08 | 0.99 ± 0.01               |

a The CD3ε⁺ cell/CD3ε⁻ cell ratio was lower for the patients than for the controls.
TABLE 2. CD3ε⁺ CD3ξ⁻, CD3ε⁺ CD3ξ⁺, CD3ε⁻ CD3ξ⁻, and CD3ε⁻ CD3ξ⁺ cells from the SMs of patients with OA and peripheral blood of healthy individuals (controls) analyzed by double immunofluorescence and flow cytometry

| Study subject | % CD3ε⁺ CD3ξ⁻ cells | % CD3ε⁺ CD3ξ⁺ cells | % CD3ε⁻ CD3ξ⁻ cells | % CD3ε⁻ CD3ξ⁺ cells |
|---------------|---------------------|---------------------|---------------------|---------------------|
| Patients      |                     |                     |                     |                     |
| 1             | 14.4                | 78.1                | 0.6                 | 6.9                 |
| 2             | 6.2                 | 81.0                | 3.3                 | 9.5                 |
| 3             | 15.3                | 63.8                | 5.4                 | 15.5                |
| 4             | 10.7                | 83.4                | 2.0                 | 4                   |
| 5             | 26.9                | 48.0                | 5.3                 | 19.8                |
| 6             | 17.9                | 51.2                | 8.9                 | 22.0                |
| 7             | 7.1                 | 84.1                | 2.3                 | 6.6                 |
| 8             | 34.4                | 25.2                | 1.8                 | 38.6                |
| 9             | 5.6                 | 71.0                | 5.8                 | 17.6                |
| Mean ± SEM    | 15.39 ± 3.27        | 65.04 ± 6.70        | 3.93 ± 0.87         | 15.60 ± 3.58        |
| Controls      |                     |                     |                     |                     |
| 1             | 7.7                 | 85.7                | 4.8                 | 1.8                 |
| 2             | 7.5                 | 86.3                | 4.0                 | 2.2                 |
| 3             | 3.9                 | 94.6                | 0.5                 | 1.0                 |
| 4             | 2.5                 | 95.2                | 1.1                 | 1.2                 |
| 5             | 2.8                 | 93.4                | 8.4                 | 5.4                 |
| 6             | 1.8                 | 95.8                | 1.3                 | 1.2                 |
| 7             | 1.1                 | 98.0                | 0.4                 | 0.5                 |
| 8             | 0.3                 | 95.0                | 0.0                 | 4.7                 |
| Mean ± SEM    | 3.92 ± 0.99         | 90.81 ± 1.99        | 2.98 ± 1.01         | 2.29 ± 0.57         |
| P value       | .0079               | .0047               | .4849               | .0058               |

nine patients with OA, revealed significantly increased proportions of CD3ε⁺ T cells compared with the proportions of CD3ξ⁻ T cells (means ± standard error of the means [SEMs], 80.48% ± 3.92% and 69.02% ± 6.51%, respectively; P = 0.0096) (Table 1). In contrast, there were no significant differences in the proportions of CD3ε⁺ and CD3ξ⁺ T cells (means ± SEMs, 94.73% ± 1.39% and 93.79% ± 1.08%, respectively; not significant) present in PBMCs from healthy donors (Table 1). The proportions of CD3ε⁻ and CD3ξ⁻ T cells in the SMs of patients with OA were significantly lower than those found in PBMCs from healthy donors (means ± SEMs for CD3ε⁺ T cells, 80.48% ± 3.92% and 94.73% ± 1.39%, respectively; P = 0.0064; means ± SEMs for CD3ξ⁺ T cells, 69.02% ± 6.51% and 93.79% ± 1.08%, respectively; P = 0.0051) (Table 1). Significant differences were also found in the CD3ξ⁺ cell/CD3ε⁺ cell ratio, which was decreased for T cells from the SMs of patients with OA compared to that for T cells from the PBMCs of healthy donors (means ± SEMs, 0.84 ± 0.06 and 0.99 ± 0.01; respectively; P = 0.0302) (Table 1).

The proportions of CD3ε⁺ CD3ξ⁻ (CD3ξ⁻) cells were significantly higher in the SMs of patients with OA than in the PBMCs of healthy donors (means ± SEMs, 15.39% ± 3.27% and 3.92% ± 0.99%, respectively; P = 0.0079) (Table 2), suggesting the presence of a sizable population of CD3ε⁺ CD3ξ⁻ cells in the SMs of patients with OA. In contrast, the proportions of CD3ε⁺ CD3ξ⁺ cells were significantly lower in the SMs of patients with OA than in the PBMCs of healthy donors (means ± SEMs, 15.04% ± 6.70% and 90.81% ± 1.99%, respectively; P = 0.0047) (Table 2). No significant differences in the proportions of CD3ε⁻ CD3ξ⁻ cells were found in the SMs of patients with OA and the PBMCs from healthy donors (means ± SEMs, 3.93% ± 0.87% and 2.98% ± 1.01%, respectively; P = 0.4849) (Table 2). Significant differences were observed in the proportions of CD3ε⁻ CD3ξ⁻ cells found in the SMs of patients with OA and in PBMCs from healthy donors (means ± SEMs, 15.60% ± 3.58% and 2.29% ± 0.57%, respectively; P = 0.0058) (Table 2), also suggesting the presence of a sizable population of CD3ε⁻ CD3ξ⁻ cells in the SMs of patients with OA. Comparison of the proportions of the CD3ε⁺ CD3ξ⁻ cells to those of the CD3ε⁻ CD3ξ⁻ cells in the SMs of patients with OA revealed significantly increased proportions of CD3ε⁻ CD3ξ⁻ cells (means ± SEMs, 15.39% ± 3.27% and 93.93% ± 0.87%, respectively; P = 0.096) (Table 2). In contrast, there were no differences in the proportions of CD3ε⁺ CD3ξ⁻ cells and CD3ε⁻ CD3ξ⁻ cells in PBMCs from healthy donors (means ± SEMs, 3.92% ± 0.99% and 2.98% ± 1.01%, respectively; P = 0.3317) (Table 2). The results of representative experiments by double immunofluorescence and flow cytometry analysis of CD3ε⁺ CD3ξ⁻, CD3ε⁺ CD3ξ⁺, CD3ε⁻ CD3ξ⁻, and CD3ε⁻ CD3ξ⁺ populations of mononuclear cells infiltrating the SMs of three patients with OA (patients 7, 3, and 4) are shown in Fig. 1. The results of representative experiments for the same cell populations from PBMCs from three healthy controls are also shown.

Amplification of the CD3ξ cDNA and CD3ε cDNA transcripts by RT-PCR showed invariably stronger bands for the CD3ε cDNA than for the CD3ξ cDNA. Representative results for 11 patients with OA are shown in Fig. 2. The CD3ξ/CD3ε transcript ratio in SMs from patients with OA (n = 11) was significantly lower than the CD3ξ/CD3ε transcript ratio in PBMCs from healthy controls (means ± SEMs, 0.063 ± 0.028 and 0.787 ± 0.023, respectively; P < 0.0001) (Fig. 3A). These results for five individuals were confirmed by semiquantitative competitive MIMIC PCR, as described previously (51) (data not shown).
The CD3ζ/CD3ε transcripts from patients with RA ($n = 9$) was also significantly lower than the CD3ζ/CD3ε transcript ratio in peripheral blood from healthy controls (means ± SEMs, 0.327 ± 0.068 and 0.787 ± 0.023, respectively; $P < 0.0001$) (Fig. 3B).

The expression of CD3ε and CD3ζ proteins was investigated by immunohistochemical staining of frozen sections of the SMs from 19 patients with OA and 11 patients with RA by using the anti-CD3ε MAb or the anti-CD3ζ MAb, respectively, as described in Materials and Methods, and the ABC indirect immunoperoxidase method. Immunoreactivity for CD3ζ was detected in the SMs of 10 of 19 patients with OA and the SMs of 9 of 11 patients with RA. The intensity of the immunoreactivity for CD3ζ, when present, was variable. As anticipated, CD3ζ protein expression was not observed in those specimens that lacked expression of CD3ζ transcripts. However, certain specimens that expressed CD3ζ transcripts (often at low levels) did not express the CD3ζ protein, suggesting the involvement, among other mechanisms, of a transcriptional mechanism in the downregulation of CD3ζ protein expression. In contrast, immunoreactivity for CD3ε was detected in all patients with OA and RA. These results are summarized in Table 3.
results of a representative immunostaining experiment are shown in Fig. 4.

**DISCUSSION**

We report here that the expression of CD3ζ/H9256 chain transcripts and protein in the SMs from patients with OA is decreased. Decreased expression of the CD3ζ chain in patients with OA may be of functional significance because the CD3ζ chain plays a significant role in antigen-specific T-cell activation (40, 62). This decreased expression of the CD3ζ chain is associated with anergy and T-cell hyporesponsiveness and may reflect chronic antigenic stimulation of T cells [12, 22, 23, 27, 31, 32, 36, 38, 43, 47, 61, 73; Pappas et al., FASEB J. 10:A1472, 1996 (abstract)]. Although such antigens have not been identified in patients with OA, evidence strongly suggesting that such an antigen-specific chronic T-cell activation may occur in patients with OA and that T cells may play an important role in the pathogenesis of the disease has been accumulating.

Mononuclear cell infiltrates consisting primarily of CD3+ T cells and monocytes (16, 19, 26, 37, 48, 51, 60) are often observed in the SMs of patients with OA (16, 19, 26, 37, 49). The T cells infiltrating the SMs of patients with OA often exhibit a nodular pattern (51, 60) and express certain early (CD69), intermediate (CD25), and late (CD45RO, HLA-DR) activation antigens. These infiltrating T cells are often angio-centric [L. I. Sakkas, C. R. Scanzello, C. D. Katsetos, N. A. Johanson, and C. D. Platsoucas, Rheumatology 39(Suppl.): 117, 2000 (abstract)], and their presence is associated with the activation of vascular endothelial cells, as determined by the expression of E-selectin (5, 20, 63).

To determine whether a specific antigen-driven clonal immune response is taking place in the SMs of patients with OA, β-chain T-cell receptor (TCR) transcripts were amplified from the SMs of patients with OA by the nonpalindromic adaptor PCR method or by Vβ-specific PCR (54, 59). The amplified transcripts were cloned and sequenced. Sequence analysis revealed substantial proportions of identical copies of β-chain TCR transcripts, suggesting the presence of oligoclonal expansions of T cells in the SMs from five of five patients with advanced OA [Scanzello et al., Arthritis Rheum. 42(Suppl.): S257, 1999 (abstract); Scanzello et al., Scand. J. Immunol. 54(Suppl. 1):59, 2001 (abstract)], strongly supporting a specific

**TABLE 3. Immunoreactivities of SMs from patients with OA or RA after staining with either anti-CD3ζ MAb or anti-CD3ε MAb**

| MAb         | No. of SM specimens positive/no. tested (%) | OA       | RA       |
|-------------|--------------------------------------------|----------|----------|
| Anti-CD3ζ   | 10/19 (52.6)                               | 9/11 (81.8) |          |
| Anti-CD3ε   | 19/19 (100)                                | 11/11 (100) |          |
| *P* value   | 0.0023                                     | 0.4583   |          |
antigen-driven immune response(s) (C. Scanzello, L. I. Sakkas, B. Xu, C. N. Johanson, and C. D. Platsoucas, submitted for publication). The antigen(s) that elicits these T-cell responses is not known. However, an autoimmune response to chondrocyte SM components has been reported in patients with OA (2). Inflammation in patients with OA may not be restricted to the joints. Perivascular lymphocytic infiltrates have been reported in muscle biopsy specimens from 18% of the patients with OA examined (67).

Incidental infections with common herpesviruses may enrich the pool of activated T cells in the SMs of patients with OA. CD8+ T cells specific for Epstein-Barr virus (EBV) and cytomegalovirus are present at an increased frequency in the SF of patients with RA (57, 65) and certain patients with OA (57, 65) as well as in the affected organs of patients with various chronic inflammatory diseases (57). However, EBV gene expression is not altered in the SMs of patients with RA or OA, despite the presence of EBV antigen-specific T-cell clones (9). The viral peptide-specific CD8+ T cells comprised approximately 4% of the total CD8+ T cells in the SF of patients with RA (11) and were oligoclonal (11). However, the clonality of the remaining T cells that did not react with these viral epitopes was not examined in that study (11). In addition, the method used to determine the proportions of these viral peptide-specific CD8+ T cells, HLA class I peptide tetramer staining, provided estimates of CD8+ T-cell frequencies that were, on average, 4.4-fold higher than the frequencies obtained by enzyme-linked immunospot assays (64). These estimates were, in turn, on average 3.3-fold higher than the frequencies obtained by limiting dilution analysis (64). Although the viral peptide-specific CD8+ T cells that are present in the SMs of these patients may exhibit decreased expression of the CD3ε antigen, their presence in relatively low proportions (see above) alone cannot account for the decreases in the proportions of CD3ε+ cells found in the SMs of patients with OA in this study.

It appears that the recruitment of virus-specific CD8+ T cells in inflammatory joints of patients with OA is chemokine driven. T cells infiltrating the SMs of patients with OA are of the TH1 type (51) and express high levels of the chemokine receptor CCR5 on their surfaces (29). The ligand of CCR5, the chemokine macrophage inflammatory protein 1α (MIP-1α), is upregulated in the SF of patients with OA (21). In the SF of patients with RA, the vast majority of virus-specific CD8+ T cells express the chemokine receptor CCR5 (11).

It is also possible that in the SMs of these patients the virus-specific T cells recognize self-antigenic epitopes as being of viral origin by molecular mimicry. Molecular mimicry is defined as the presence of epitopes with substantial structural homology between host proteins and the proteins of microorganisms such as viruses, bacteria, and other organisms (13, 41, 42, 69). Therefore, a host immune response against a viral epitope may recognize as non-self a host epitope with substantial structural homology, even after the microorganism that elicited the immune response is not present. These molecular mimicry responses may lead to the development of autoimmune disease. Along these lines, it has been reported that T cells specific for an EBV antigen also respond to a self-peptide from a serine-threonine kinase (34). Intermolecular or intramolecular epitope spreading is another mechanism that can lead to the development of T-cell responses in the SMs of patients with OA (7, 24, 25, 58, 71). Epitope spreading is defined as the generation of de novo immune responses to new self-antigenic epitopes which are different and which do not share structural homology (cross-reactivity) to those that elicited the initial immune response. Epitope spreading is generated in the environment of chronic inflammation. Epitope spreading was first identified in autoimmune demyelinating diseases of the central nervous system (1, 24, 25, 42, 58, 71) and is now identified in antitumor immunity (10) and the immune response against organ grafts (7).

The SMs of patients with OA exhibit a TH1 cytokine profile (51, 52, 60). It could be argued that this TH1 response may be driven primarily by nonspecific functions of macrophages instead of T-cell responses to a specific antigen(s). Macrophages and synoviocytes produce interleukin-12 (52), a potent cytokine that drives the TH1 immune response by inducing the production of proinflammatory cytokines. Increased concentrations of macrophage inflammatory protein-1α (MIP-1α) have been reported in the SF of patients with OA (21). MIP-1α is a ligand for the chemokine receptor CCR5, which is expressed on TH1 cells (29). In a recent review, Pelletier et al. (46) proposed that OA is an inflammatory disease and cited macrophages as the exclusive source of inflammation in the pathogenesis of OA, ignoring the role of T cells (46, 55). Although the mononuclear cell infiltration and the TH1 response may be explained by a nonspecific activation of T cells, such as cytokine-induced activation, our findings (see above) of T-cell oligoclonality in the SMs of five of five patients with advanced OA [Scanzello et al., Arthritis Rheum. 42(Suppl.): S257, 1999 (abstract); Scanzello et al., Scand. J. Immunol. 54(Suppl. 1):59, 2001 (abstract)] strongly suggest that T cells have undergone antigen-driven proliferation and clonal expansion in situ in the SMs of patients with OA in response to an as yet unidentified antigen(s). Although the nature and characteristics of the antigen(s) remain to be determined, it is clear that the view traditionally held by many rheumatologists and scientists alike that OA is a noninflammatory disease should be abandoned and that the disease should be reclassified.

In a manner similar to that for other conditions characterized by chronic T-cell activation (22), such as RA (31, 32), SLE (27), lepromatous leprosy (73), human immunodeficiency virus infection (66), renal carcinoma (12), colorectal carcinoma (36, 38), ovarian carcinoma [23; Pappas et al., FASEB J. 10:1472, 1996 (abstract); Pappas et al., J. Allergy Clin. Immunol. 99: S447, 1997 (abstract)] and Hodgkin's disease (47), T cells from the SMs of patients with OA exhibit decreased expression of CD3ε chain transcripts and protein. Although studies with T cells from the SMs of patients with OA have not been reported, this decreased expression of the CD3ε chain in these other diseases is associated with anergy and T-cell hyporesponsiveness. In patients with RA, T cells from the peripheral blood and the SMs are functionally deficient or "frustrated," although these T cells express early, intermediate, and late activation antigens (19, 51). Additionally, these T cells from patients with RA exhibit decreased in vitro proliferation and impaired increases in intracellular Ca2+ levels after stimulation with mitogens and recall antigens (for a review, see reference 1). Similarly, in TILs [23, 28; Pappas et al., FASEB J. 10:1472, 1996 (abstract)], peripheral blood T cells of patients infected with human immunodeficiency virus (66), and tumor-
bearing mice (40), the decreased expression of CD3ζ was associated with reduced T-cell proliferative responses and decreased phosphorylation of the CD3ζ chain [23, 28; Pappas et al., FASEB J. 10:A1472, 1996 (abstract)]. Decreased CD3ζ chain expression in T cells from the SF of patients with RA was accompanied by decreased phosphorylation of the CD3ζ chain (32). Decreased phosphorylation of the CD3ζ chain was also observed in antigen-induced T-cell death (43) and in T cells that are in anergic state (30, 33).

The hyporesponsiveness of T cells in patients with RA could also be attributed to chronic exposure to tumor necrosis factor alpha (TNF-α), since it was reversed in vivo by anti-TNF-α antibody treatment (8). The effect of anti-TNF-α antibody treatment on CD3ζ chain expression was not investigated in that study (8). Apart from a chronic antigenic stimulation, other factors may also contribute to decreased CD3ζ chain expression. For example, activated macrophages can induce decreased CD3ζ chain expression in T cells by cell contact-dependent interaction (4) or oxidative stress (44).

The defect in CD3ζ chain expression appears to be reversible, and it was restored in TILs after stimulation with interleukin-2 [70; Pappas et al., J. Allergy Clin. Immunol. 99:5447, 1997 (abstract)]. Similarly, CD3ζ chain expression was restored in peripheral blood T lymphocytes in patients with Hodgkin’s disease after stimulation with anti-CD3 and anti-CD28 MAbs (47). In patients with SLE, the loss of CD3ζ chain expression was found to be associated with an increased frequency of alternative splicing of the CD3ζ chain RNA (39). However, one study reported that the CD3ζ chain can serve as a substrate for caspase 3, and the decreased expression of the CD3ζ chain was associated with apoptosis of TILs (14). Furthermore, two populations of T cells, CD3ζ+CD3ζ− cells and CD3ζ CD3ζ− cells, were found to be present in substantial proportions (about 15%) in the SMs of patients with OA. These two populations of T cells are virtually absent from the peripheral blood of healthy donors. Their function is not known at present, and it is not known whether these populations can differentiate to CD3ζ− CD3ζ− T cells.

In conclusion, our finding of decreased expression of the CD3ζ chain by T lymphocytes from the SMs of patients with OA is suggestive of a chronic antigenic T-cell stimulation and reinforces the concept of T-cell involvement in OA.

We thank Norman Johanson (formerly of the Department of Orthopedics, Temple University School of Medicine, and presently of the Department of Orthopedics, Hennemann University, Philadelphia, Pa.) for providing specimens for this study.

This work was supported in part by grant T32 AI07101 from the National Institutes of Health.

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