IDENTIFICATION OF DENDRITIC CELLS AND MESENCHYMAL STEM CELLS IN THE SYNOVIAL FLUID OF OSTEOARTHRITIC PATIENTS

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INTRODUCTION:
Osteoarthritis (OA) is the most common form of joint disease; however its etiology is still unclear. OA pathogenesis is characterized by cartilage breakdown due to repetitive trauma and damage to the articular cartilage and concomitant hypertrophic reactions in the bone compartment. Chondrocytes and synoviocytes release various cellular products, which induce remodeling of surrounding tissue by recruiting immune and nonimmune cells to the site of injury. One such cell type, the dendritic cell (DC), is found in the synovial fluid (SF) of OA patients. There is evidence that various DC subsets exist within the SF, but their role in the pathogenesis of OA remains to be established. Mesenchymal stem cells (MSC) have also been identified in SF but in very low numbers. MSCs can differentiate along various lineages and have the potential to modulate immune responses. In view of the above, we analyzed the cell populations in OA SF by flow cytometry to better characterize the relevant cell populations.

METHODS:
Patients: Twelve patients with OA (9 women and 3 men) participated in this study, all of which were undergoing total knee replacement surgery at the Brigham and Women’s Hospital. Their average age was 64 years with a range of 58 to 87 years. OA was diagnosed based on clinical and radiological findings in accordance with the criteria of the American College of Rheumatology.
Sample collection: SF specimens were collected in heparinized plastic syringes and immediately placed at 4°C until processed. For processing, samples were diluted 1:10 in phosphate buffered saline (PBS). Cells were sedimented by centrifugation (1500 x g for 10 min), collected, and frozen in the presence of 5% DMSO until characterized by flow cytometry.
FACS analysis: For FACS analysis, cells were cultured in blocking buffer for 30 minutes at 4°C and aliquoted into 96-well micro titer plates at a concentration of 1 x 10^6 cells/well and stained with the following mAbs: CD2, CD3, CD4, CD8, CD14, CD19, CD33, CD45, CD56, CD123, CD133, and HLA-DR. After incubating with the above mAbs for 30 minutes at 4°C, samples were washed with PBS and fixed with 1% paraformaldehyde. Samples were acquired using a FACSArray (BD) and data evaluated using FlowJo software.

RESULTS:
The percentages of total T cells (CD3), T helper cells (CD4), cytotoxic T cells (CD8), together with those for MSC (CD133), and DCs (CD2, CD3, and HLA-DR) in the SF of OA patients are depicted in the Figure. The percentage of total T cells was 7.2 ± 3.6% and that of CD4 and CD8 T cells was 4.6 ± 2.4 and 2.5 ± 1.2%, respectively. These values are markedly lower than those seen in the peripheral blood or SF from patients with rheumatoid arthritis (RA) (1). As for other lymphocyte subsets, the percentages of B and NK cells were 9.3 ± 6.7% and 4.5 ± 5.4%, respectively. These values are well within the range found in peripheral blood. CD133 antigen was used to identify the mesenchymal stem cell population and found to be present on 11 ± 5.4% of the total SF cells. Dendritic cells, defined by the presence of CD2, CD33, and HLA-DR surface antigens (2), represented 32.4 ± 14.4% of the SF cell population and values ranged from 12 to 55%. Further, DC analysis for the expression of the plasmacytoid marker, CD123, revealed that 19.4 ± 8.9% of the DCs expressed this antigen, with values ranging from 9 to 40%. A subset of these CD123+ DCs (7.3 ± 3.9%) expressed CD2, CD133, and HLA-DR at extremely high levels (mean fluorescent intensities = 10^4). In every OA SF sample analyzed, both CD133+ cells and the DCs were found present at greater percentages than T, B, or NK cells.

DISCUSSION:
This study examines the cell populations present in the SF of OA patients. We report sizeable dendritic cell and mesenchymal stem cell populations in the OA SF. Remarkably, in each sample analyzed, the DC population represented the majority of the immune cells present. The much lower percentage of plasmacytoid DC reported in a previous study is likely due to isolation procedure, which includes enzymatic digestion, density gradient separation, and cell surface markers used for immunobead positive-selection (3). Our single step procedure and the use of the three robust cell surface markers, CD2, CD33, and HLA-DR, optimizes cellular yield and identification. As to the presence of MSC, we used CD133 as a marker because previous reports have shown that it identifies stem cells and is not present on terminally differentiated cells. CD133+ cells can differentiate along mesenchymal and hematopoietic pathways (4). Using CD133 in conjunction with our SF isolation method, we observed a population of approximately 11% MSC. This value is much higher than that previously reported for SF (5). However, this percentage is comparable to that reported in synovial tissue of OA patients (6). This elevated number of MSCs may be instrumental in inhibiting the development of autoimmune disease related to chronic inflammation. A previous in vitro study supports this hypothesis. It showed that MSCs interact with DCs and inhibit DC1-mediated immune responses and augment DC2-mediated immune responses (7). In view of these findings, the detailed mechanism by which MSC modulate the immune response in OA requires further investigation.

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