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Effects of hyaluronic acid (HA) viscosupplementation on peripheral Th cells in knee and hip osteoarthritis

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SUMMARY

Objective: Determine Th lymphocytes concentration in patients with knee or hip osteoarthritis (OA). Evaluate their change after HA viscosupplementation.
Methods: Patients with early primary knee or hip OA (ACR Criteria) were recruited in two groups: group A was only observed longitudinally, group B was treated with a course of three weekly intra-articular injections of HA. A healthy control group gender and age matched was enrolled too. All subjects were followed for 3 months. Flow cytometry was performed from blood samples to assess T cells sub-populations (CD3, CD4, CD8, CCR6, CD38, CxCR3, HLA DR) at baseline and at 3-months visit.
Results: 86 patients were recruited with OA: 49 in Group A (35 knee OA, 14 hip OA), 37 in Group B (24 knee OA, 13 hip OA). 23 in Control Group. Activated CD4 T cells ($CD4^+CD38^+DR^+$, $CD4^+CD38^-DR^+$), Th2 ($CD4^+CXCR3^-CCR6^-$),Th1 ($CD4^+CXCR3^+CCR6^-$) were higher at baseline in group A and B than in control group. After the HA course activated T cells were lower in group B than in group A ($P = 0.01$). Th17 ($CD4^+CXCR3^-CCR6^+$) at baseline were higher in groups A and B than in control group and decreased levels in Group B after the HA course were observed ($P = 0.03$).
Conclusion: The presence of activated T cells in patients with OA confirm that OA is a disease with an immunological/inflammatory involvement. Our preliminary results seems to show that HA injections could lower the levels of activated T cells, and so regulate the articular milieu.

Introduction

Osteoarthritis (OA) is a chronic, degenerative joint disease characterized by the progressive destruction of articular cartilage, joint space narrowing, subchondral bone remodeling, joint marginal osteophyte formation and synovitis. OA causes joint pain, stiffness, swelling and reduced range-of-motion having a serious impact on health related quality-of-life, showing several characteristics with rheumatoid arthritis, including joint destruction and synovitis.

OA can be diagnosed by the presence of joint irregularities and deformities on X-radiographic images. The grade of joint degeneration reflects disease severity. Non-invasive biochemical analyses have been developed to evaluate disease progress and severity, and provide a nonradiographical alternative for the early detection of OA. Many factors contribute to an increase risk of OA and include obesity, genetics, aging, and trauma to the joint.

Although the etiology and pathophysiology of OA are both poorly understood, it is believed that secreted inflammatory molecules (such as proinflammatory cytokines and adipokines) are among the critical mediators of the disturbed processes implicated in OA pathophysiology. Humoral and cellular immunity, both innate and adaptive immune response, are known to be involved[1]. In particular, in many studies on OA, it has been demonstrated that an inflammatory synovium/synovitis has linked to increased cartilage damage and pain[2,3]. Synovial tissue of OA patients show infiltrates of immune cells including T-cells, B-cells and macrophages. Immunoglobulins and immune complexes against cartilage components are detected in cartilage, synovium and plasma in OA patients[4,5]. Finally, key role of complement activation in OA synovium has been identified[6].

From data literature, $CD4^+$ T lymphocytes (particularly Th17 subtype) and their cytokines have been reported to play a major

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role to activate rheumatic diseases inflammation as rheumatoid arthritis or OA. In a proinflammatory milieu, chondrocytes become metabolically active and initiate inflammatory processes that degrade articular cartilage and subchondral bone. Chondrocytes secrete several inflammatory cytokines that work synergistically to stimulate synthesis of enzymes that break down cartilage. Normally, synovial fluid contains high levels of hyaluronic acid (HA, a polysaccharide produced by the chondrocytes and synoviocytes) that help to maintain high fluid viscosity and the normal integrity of the joint by attenuating inflammation and preserving the normal cartilaginous matrix. In OA, the synovial fluid viscosity and elasticity are decreased. While HA may help to lubricate and cushion the joint, it can help maintain cartilage matrix and minimize inflammation. In OA, the molecular weight and concentration of HA are reduced, thereby lowering fluid viscosity and elasticity. Protection against articular injury is compromised and OA damage ensues. Intra-articular injections of HA (i.e., viscosupplementation) are approved worldwide for the treatment of pain associated with OA of the knee. In addition to a purely mechanical effect due to the viscosity of the products, intra-articular HA viscosupplementation is thought to provide a range of biological actions including anti-inflammatory effect. *In vitro* data suggest that supplemental HA can suppress IL-1 production, and may increase synovial fluid viscosity[7]. We hypothesize that intra-articular HA can suppress not only the local intra-articular proinflammatory milieu, but also can reduce the overall inflammatory cytokine response. Primary purpose of this study was to evaluate the circulating levels of activated $CD4^+$ and $CD8^+$ lymphocytes in patients with OA, comparing with healthy control. Secondary purpose was to evaluate the changes in lymphocytes after 3 months from an intra-articular HA injection course and the effectiveness in terms of variation of Lequesne Pain-functional index.

Methods

Patients

Patients with hip or knee OA were recruited from the Rheumatology Unit Fornaroli Hospital from January 2012 to October 2013. The inclusion criteria were a diagnosis of bilateral knee or hip OA according to ACR Criteria, with a the Kellgren–Lawrence score of 2–3 and Pain VAS at least 50 mm on a 0–100 mm visual scale. The exclusion criteria were: presence of rheumatoid arthritis or other rheumatic diseases, pregnancy, allergic to hyaluronans, currently experiencing a knee infection or skin infection around the injection site. An age, sex and BMI matched control group was screened for OA and, after exclusion of OA diagnosis, they were recruited too between blood donors of our Hospital; this group didn't meet hip or/and knee OA ACR criteria. Patients with diffuse OA to many joints (e.g., OA of knee associated with hands OA or wright OA or spine OA) were excluded. Weight bearing anteroposterior knee and hip anterior-posterior radiographs were classified according to the Kellgren–Lawrence (KL) radiographic rating scale. All patients, inclused controls, read, understood and signed an informed consent and compiled a pain-functional Lequesne Index. The trial was conducted in accordance with the ethics principles of the Declaration of Helsinki and was approved by the local research ethics committees.

Analgesics, non-steroidal anti-inflammatory drugs (NSAIDs), symptomatic slow acting drugs for OA (SYSADOAs) or disease modifying (DMOADs) if taken before entering the trial were not modified throughout the period of the study. Patients with OA longitudinally evaluated were enrolled in Group A (group A1 with 35 knee OA and group A2 with 14 hip OA) and no intra-articular treatment was performed in these groups. Patients with knee OA or hip OA treated with an HA intra-articular course were recruited in Group B (B1: 24 knee OA and B2: 13 hip OA). 23 subjects were enrolled finally as control-healthy group.

Knee intra-articular injection procedure

The patient sat with the extended knee(s). An anteromedial or lateral approach was performed for these injections. A 20 g needle was used and a syringe of 2 ml of viscosupplement was then delivered. We used for viscosupplementation sodium hyaluronate with a molecular weight of 800–1200 kDa (Sinovial Forte® 1.6% 2 ml IBSA). Two more injections were provided to each patient at weekly intervals for a total of three injections.

Hip eco guided intra-articular injection procedure

Patients were examined supine, with the hip internally rotated by 15–20°. A 10 MHz linear transducer had a sterile device for biopsy attached. Ultrasound scans of the hip joint were taken on an anterior parasagittal axis, lateral to the femoral vessels. The probe was aligned along the long axis of the femur neck to visualize both the acetabulum and the femoral head. Using an anterosuperior approach, a G20 (9 cm) spinal needle was inserted through the biopsy guide into the joint capsule, until the femoral head was reached. Using real time imaging software, the needle was inserted until it was visualized in the articular recess and so HA was injected. A course of three injections with Sinovial Forte® was performed for all patients with hip OA in the group B2.

Flow cytometry

Freshly drawn EDTA blood samples were analyzed by 8-color flow cytometry (FACSCanto II, Becton Dickinson, Milan) with the following conjugated antibody panel: CD45-FITC; CXCR3-PE; CD4-PerCP-Cy5.5; CCR6-PE-Cy7; CD38-Alexa 647; CD8-APC-H7; CD3-V450; HLADR-V500 at the appropriate concentrations (all from Becton Dickinson). After 20-min staining in the dark, 2 ml of ammonium chloride lysing was added for 10 min. After centrifugation at 1500 rpm for 7 min, the pellet was resuspended in 200 μL of cold PBS and immediately analyzed.

At least 50,000 lymphocytes (defined as $CD45^{+++}$, SSClow cells) were acquired.

The gating strategy included the parallel capture of $CD4^+/CD3^+$ and $CD8^+/CD3^+$ cells in two separate downstream hierarchies. Each parent subset was then further dissected into functional subpopulations, namely $CD4^+$ T cells as Th1 cells ($CD4^+$ $CXCR3^+$ $CCR6^-$), Th2 cells ($CD4^+$ $CXCR3^-$ $CCR6^-$) and Th17 cells ($CD4^+$ $CXCR3^-$ $CCR6^+$), respectively according to Maecker *et al.*, 2012[8]. Both $CD4^+$ and $CD8^+$ cells were divided into quiescent ($CD38^-$ $HLADR^-$) or activated elements ($CD38^+$ and/or $HLADR^+$). Functional subset percentages were calculated over the total lymphocyte population and over the parent $CD4^+$ or $CD8^+$ subsets, respectively, and all values were also recorded as absolute levels per microliter on the basis of total lymphocyte count (Beckman Coulter DXH800, Milan).

Statistical analysis

All the variables collected were normally distributed as stated by Shapiro–Wilk test. Homoscedasticity of variances was assessed with Cochran/Hartley or Levene tests. Continuous variables were presented as mean SD, and compared using two-tailed Student's *t*-test or one-way analysis of variance (ANOVA). Links between continuous variables collected in the groups during the study period were estimated with MANOVA for repeated measures

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