GRANULOCYTE-MACROPHAGE COLONY STIMULATING FACTOR AND ITS RECEPTOR CD116 EXPRESSION IN THE SYNOVIAL OF OSTEARTHROSIS PATIENTS IS NEGATIVELY CORRELATED WITH PAIN


Purpose: Although pain is the most predominant symptom in osteoarthritis (OA), the underlying mechanisms are poorly understood. The synovium is a rich source of many mediators that play a role in OA and changes in synovitis have been associated with fluctuation of knee pain. The pro-inflammatory cytokine granulocyte macrophage-colony stimulating factor (GM-CSF) was recently shown to be correlated with inflammatory synovitis in an animal model of RA, and is implicated to play a role in pain in OA. The present study evaluates the role of GM-CSF and its receptor CD116 in the synovium of OA patients in relation to OA pain.

Methods: Synovial tissue was collected of a cohort of end-stage knee OA patients (n = 18, mean age 69.5 years, 61% female) selected for total knee replacement surgery. These patients didn’t use medication in the year prior to surgery, except for commonly acetaminophen. Expression of GM-CSF and CD116 in the synovial sublining and lining or total was analysed using frozen section immunohistochemistry (IHC-f). To assess pain levels, the Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) questionnaire (100 = no pain, 0 maximum pain) and the Visual Analogue Scale (VAS) were used. Histology and macroscopy of the synovium were scored using the modified Goldberg and Cohen score and synovial inflammation was graded in three categories: colour, angiogenesis, and fibrillation. Additionally, ex vivo TNFa, IL-1b, PGE2 and NO levels of the synovial tissue were measured.

Results: The patients had an average WOMAC score of 55.3 (+17.9) and a VAS score of 30.3mm (+20.0). GM-CSF and CD116 were both expressed in the sublining and lining of the synovial tissue (on average resp. 424 and 3773 positive cells/mm2 and 948 and 3287 positive cells/mm2 for GM-CSF and CD116 respectively). A negative correlation was observed between clinically knee pain (WOMAC) and synovial sublining (r = -0.613, p = 0.007), synovial lining (r = -0.130, p = 0.581) and total (r = -0.520, p = 0.027) GM-CSF expression. Similarly, a negative correlation was found for its receptor CD116 expression versus pain (WOMAC) in the synovial sublining (r = -0.663, p = 0.003), lining (r = -0.520, p = 0.027) and the total score (r = -0.668, p = 0.002). So a high pain level was significantly associated with low expression of GM-CSF and CD116.

Conclusions: Undergoing joint surgery results in a decrease of GM-CSF expression, which might contribute to healing. Clearly, more research is warranted before targeting GM-CSF in the synovium is a realistic option to improve pain in OA.

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ESTABLISHING A METHOD FOR MEASURING PRIMARY KNEE HYPERALGESIA IN THE MURINE DMM MODEL OF OSTEOARTHRITIS

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Purpose: The purpose of this study was to establish a method for measuring primary knee hyperalgesia using the murine DMM model of experimental osteoarthritis.

Methods: DMM or sham surgery was performed in the right knees of 10-week old male C57BL/6 mice (n = 5-14/time point). At 0 (pre), 2, 4, 6, 8, or 16 weeks post surgery, knee hyperalgesia was assessed using a Pressure Application Measurement (PAM) device from Ugo Basile by two independent blinded users. Mice were restrained by hand and the PAM device was used to press against either the ipsilateral or contralateral knee. The PAM software guided the user to apply an increasing amount of force at a constant rate (30 g/s), up to a maximum of 450 g. If the mouse tried to withdraw its knee, the force at which this occurred was recorded. If the mouse did not try to withdraw, the maximum possible force of 450 g was assigned. Two measurements were taken per knee, one on the medial side, and one on the lateral side, and the withdrawal force data were averaged. In addition, the data were analyzed in a binary fashion using the chi-square test in which a mouse was assigned to either withdrawal or no withdrawal categories, based on whether or not the mouse tried to withdraw its knee on either the medial or the lateral side.

Results: Prior to DMM surgery, 23% of naïve mice responded to force applied to the ipsilateral knee, with the mean withdrawal force of 442 g. At 2, 4, and 6 weeks post surgery, the majority of both DMM and sham-operated mice responded to force applied to the operated knees, with no significant difference seen between treatments (Fig 1). By 8 weeks post surgery, 86% of DMM mice were still withdrawing their ipsilateral knees, while only 40% of sham mice responded (p = 0.08). Finally, at 16 weeks post surgery, when the DMM mice have moderate to severe joint damage, 50% of DMM mice responded to force on their ipsilateral knee, while sham mice had returned to baseline (i.e., 25% responding).

Next, we examined the effect of the treatments on the magnitude of the withdrawal forces. At 2 and 4 weeks post surgery, both DMM and sham mice responded to similar forces on their ipsilateral knees (290-336 g), significantly decreased from baseline (p < 0.0001). At 6 weeks post surgery, the forces at which both sham and DMM mice responded had significantly increased to 402 g and 380 g, respectively, and this remained the same through 8 weeks post surgery, with no differences between the two treatments (p = 0.05 vs baseline). At 16 weeks post surgery, the withdrawal forces had increased for both treatments to 412 g, no longer significantly different from baseline. Interestingly, applying force to the contralateral knee at 2 and 4 weeks post surgery provoked withdrawal responses in both DMM and sham mice, in similar numbers of mice and at similar withdrawal forces, when compared to the ipsilateral responses. At 6-16 weeks post surgery, less than 25% of mice responded to force applied to the contralateral knee, with no differences seen between DMM and sham mice.

Conclusions: PAM provides a promising technique to assess primary knee hyperalgesia associated with OA. From these pilot feasibility studies, performed by two independent blinded evaluators, the following observations can be made: Primary knee hyperalgesia peaked during the post-surgical phase (up to 4 weeks post DMM or sham surgery). By 16 weeks post surgery, 50% of DMM mice continued to withdraw their knees, while sham mice returned to baseline levels, suggesting that structural joint damage may play a role in prolonging knee hyperalgesia.

Finally, bilateral hyperalgesia was detected during the post-surgical pain period, while the chronic phase of the disease was associated with hyperalgesia in the ipsilateral knee only.
THE NEUROHISTOLOGY OF PAINFUL AND PAIN-FREE ROTATOR CUFF TENDONS: A CASE CONTROL STUDY

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Purpose: Our main purpose was to compare the tendon neurohistology in two groups of patients; one with significant pain prior to undergoing SAD and one with resolved pain over 6 years post SAD.

Methods: Supraspinatus tendon specimens were obtained using an ultrasound guided biopsy technique from 9 patients with painful RCT resistant to conservative management (painful group) and 9 pain-free patients at over 6 years (median years 6.4 months) following SAD (pain-free group). Pain symptoms were measured using the validated Oxford Shoulder Score (OSS). Structural tendon integrity was assessed ultrastructurally. The tendon tissue was analysed using basic histological techniques (Haematoxylin and Eosin, and Acanth Blue) and Immunohistochemistry. Image analysis was performed by two blinded observers using ImageJ to quantify the amount of 3,3′-diaminobenzidine staining present. Isotype controls were also processed. Mann-Whitney U tests were carried out using SPSS with significance levels set at a minimum of p < 0.05.

Results: The groups were similar in terms of age, sex and structural tendon abnormalities. The painful group consisted of 7 males and 2 females, the pain-free group of 6 males and 3 females. The mean age of the painful group was 50 years (range 38 to 61) and that of the pain-free group was 53 years (range 39 to 65). The median OSS in the painful group was 32 (range 23 to 34) and this was significantly lower (p = 0.0002) than the median OSS in the pain-free group (all 48). There were two partial thickness tears in both groups and no full thickness tears. The modified Bonar scores in the painful and the pain-free groups were comparable. There were no significant differences between groups in terms of cellularity, vascularity, proliferation and hypoxia inducible-factor 1α expression. The leucocyte count (CD45 positive cells) and macrophage count (CD68 positive cells) were increased in the painful group versus pain-free (p = 0.01 and 0.002 respectively). The expression of the metabotropic glutamate receptor 7 (mGluR7) was reduced in the painful group versus pain-free (p = 0.008 and 0.002 respectively). PGF 2α (a nerve marker) expression was increased in the painful group versus pain-free (p = 0.008). There were no significant differences in glutamate, the inotropic glutamate receptor (NMDAR1) and the metabotropic glutamate receptors (mGluR1, 2 and 5) between groups.

Conclusions: This study has shown that specific characteristics of tendon histology are associated with a resolution of shoulder pain over six years following SAD. This provides strong evidence that the rotator cuff tendon is of key importance in the symptomatology of RCT. The mechanism behind these tendon differences remains unclear. These findings are novel and improve our understanding of pain in RCT, and may help provide novel therapeutic targets.

NEGATIVE CORRELATION BETWEEN THE POPULATION OF CD105 POSITIVE SUBSET OF MONOCYTES/MACРОPHAGES IN SYNOVIAL FLUID AND THE SEVERITY OF JOINT PAIN AFTER ANTERIOR CRUCIATE LIGAMENT RECONSTRUCTION SURGERY


Purpose: Synovial fluid is an interstitial fluid secreted by fibroblastic cells in the synovial membrane. The physiological functions of synovial fluid include reduction of friction, shock absorption, nutrient, and waste transportation in the joint. In addition, previous studies showed that mesenchymal stem cells (MSCs), which are considered to contribute the tissue regeneration, reside in synovial fluid and the number of these cells increased after joint injury. These data strongly suggest that both the contexts of cytokines and cellular components in synovial fluid may greatly influence the recovery process after joint injury or surgery. In this study, we aimed to analyze the dynamic changes of cellular components in synovial fluid after anterior cruciate ligament reconstruction surgery (ACL-R) and compared them with the clinical conditions such as joint pain of each patient. Here we report that the population of CD105+ subset of Monocytes/Macrophages negatively correlates with the severity of joint pain in the early stage of recovery process after ACL-R.

Methods: This study was approved by the Ethics Committee of Tokyo Medical and Dental University. All patients enrolled in this study, who underwent ACL-R from March till July 2013 in our university hospital, gave their full, written, informed consent for participation prior to the operative procedure (16 cases, Male:11, Female:5, 13-44 year-old, Median:21.5 year-old). Synovial fluid was obtained at day 4 to 5 after surgery and cellular components were analyzed by flow-cytometry (BD FACS Verse®). To evaluate the severity of joint pain semi-quantitatively, we collected Numerical Rating Scale (NRS) Visual Analog Scale (VAS) of each patient at day 4 to 5 after surgery (severity of joint pain when the patient woke up). Pearson’s correlation coefficient test was employed for the statistical analysis and values of p < 0.05 were considered significant.

Results: Flow-cytometric analyses detected CD3+ T cells, CD56+ NK cells, CD68+ Macrophages, and CD44+CD73+CD90+CD105+MSCs in the synovial fluid at day 4 to 5 after ACL-R, although the population of each cell varied in patients. Population of CD9+ B cells was almost undetectable in all the patients analyzed. Pearson’s correlation coefficient test revealed that VAS was negatively correlated with the population of CD105+ cells in synovial fluid (r = -0.52, p < 0.05). Since we did not observe significant negative correlation between VAS and the population of CD44+CD73+CD90+CD105+ cells, those are less than 10% of total CD105+ cells, we speculated that the CD105+ cells those do not have characteristics of MSCs may have roles in joint pain. Further analyses indicated that CD105+ cells did not co-express CD3, CD56, and CD68. Moreover however 90% of CD105+ cells co-expressed CD11b and CD14, suggesting that these cells are a subset of Monocyte/Macrophages. Positive rate of CD105 in total CD11b+CD14+ cells was almost 10%.

Conclusions: Here we showed that the population of CD105+ subset of Monocytes/Macrophages was negatively correlated with the severity of joint pain after ACL-R. We expect that the functional analyses of these cells may give us information to understand the molecular mechanisms of joint pain.

ALTERATIONS IN CENTRAL PAIN PROCESSING ARE NOT RESTRICTED TO END STAGE OSTEARTHROPATHY IN THE MONOSODIUM IODOACETATE MODEL

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Purpose: Osteoarthritis is a group of conditions that causes chronic pain and disability. Mechanisms of central sensitization contribute to the manifestation of aberrant pain responses such as mechanical allodynia and spread of pain beyond the area of tissue damage. Although evidence for central facilitation of pain has been noted in patients undergoing joint replacement for OA, its contribution to early, less severe OA is uncertain. OA severity can be evaluated by the macroscopic appearance of articular surfaces, or microscopic changes in tissue sections. It remains unclear which pathological features mediate the manifestation of aberrant pain responses such as mechanical allodynia and spread of pain beyond the area of tissue damage.

Methods: Male Sprague-Dawley rats (n = 8/group, 330–450g) were anaesthetised and given a single intra-articular injection of low (0.1 mg) and standard doses (1 mg) of monosodium-iodeacetate (MIA) in the rat and to investigate associations between structural features of OA and pain responses.

Results: Flow-cytometric analyses detected CD3+ T cells, CD56+ Natural Killer (NK) cells, CD68+ Macrophages, and CD44+CD73+CD90+CD105+MSCs in the synovial fluid at day 4 to 5 after ACL-R, although the population of each cell varied in patients. Population of CD9+ B cells was almost undetectable in all the patients analyzed. Pearson’s correlation coefficient test revealed that VAS was negatively correlated with the population of CD105+ cells in synovial fluid (r = -0.52, p < 0.05). Since we did not observe significant negative correlation between VAS and the population of CD44+CD73+CD90+CD105+ cells, those are less than 10% of total CD105+ cells, we speculated that the CD105+ cells those do not have characteristics of MSCs may have roles in joint pain. Further analyses indicated that CD105+ cells did not co-express CD3, CD56, and CD68. Moreover however 90% of CD105+ cells co-expressed CD11b and CD14, suggesting that these cells are a subset of Monocyte/Macrophages. Positive rate of CD105 in total CD11b+CD14+ cells was almost 10%.

Conclusions: Here we showed that the population of CD105+ subset of Monocytes/Macrophages was negatively correlated with the severity of joint pain after ACL-R. We expect that the functional analyses of these cells may give us information to understand the molecular mechanisms of joint pain.