Brief Report

Acute inflammation with induction of anaphylatoxin C5a and terminal complement complex C5b-9 associated with multiple intra-articular injections of hylan G-F 20: a case report

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SUMMARY

Objective: The purpose of this case report was to investigate local immune mechanisms present during an acute inflammatory flare initiated by viscosupplementation with hylan G-F 20 in a patient with osteoarthritis (OA) and past meniscectomy.

Experimental design: A patient with a history of bilateral OA and partial left knee meniscectomy, who had received three injections of hylan G-F 20, was diagnosed with an acute flare reaction in the left knee. Her chart was evaluated for clinical, radiological, and laboratory findings and for clinical follow-up. Histopathological synovial examination and real-time polymerase chain reaction (RT-PCR) for genes with major roles in local inflammation and enzyme-linked immunosorbent assays (ELISAs) for markers of complement activation and cytokines were performed. To study the impact of the inflammatory and immune features we compared the case patient with groups of three representative OA and three rheumatoid arthritis (RA) patients.

Results: The patient exhibited evidence of highly increased acute phase reactant C-reactive protein (CRP) in the blood. The pathological examination of the synovial membrane identified abundant fibrinous exudate with numerous particles of hyaluronan surrounded by a dense infiltrate of neutrophils and eosinophils. The synovium had moderate hypertrophy and sclerosis as well as an inflammatory infiltrate predominantly composed of T lymphocytes and macrophages with scattered perivascular eosinophils and neutrophils. Immunoperoxidase staining identified numerous deposits of C5b-9 in the fibrinous exudates and the synovial membrane of the patient. Similar findings were observed in the RA patients, whereas deposits were rare in OA synovial samples. In addition, both anaphylatoxin C5a and the terminal complement complex C5b-9 were present at high levels, comparable to those in RA patients. The levels of mRNA for interleukin-1 beta (IL-1β), IL-6, and the neutrophil marker myeloperoxidase (MPO) were markedly increased compared to those in the RA and OA patients.

Conclusions: This present study is indicative of a pseudo-septic acute inflammatory reaction in response to local accumulation of hylan G-F 20 with the activation of complement and local invasion of pro-inflammatory cells.

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Introduction

Hylan G-F 20 (Genzyme Biosurgery, Cambridge, MA) is a safe and generally well-tolerated viscosupplementation for intra-articular injections in patients with symptomatic osteoarthritis (OA)1,2 as well as after arthroscopic meniscectomy3. Severe local flares have been described as rare reactions in patients with bilateral OA and a history of unilateral meniscectomy4. While distinctive inflammatory flares were previously described, a clear mechanism
responsible for their initiation has yet to be studied in detail. Hylan G-F 20 gains immunogenicity by chemical cross-linked processing that enables it to persist longer when injected in the knee. In addition, in patients who underwent meniscectomy, cytokine-driven synovial inflammation occurs more frequently than in patients with established knee OA. In these patients specifically, the combination of the two may be one of the possible risk factors involved in the pathogenesis of the flare-ups.

In our case report, we provide histological and immune-biochemical evidence to suggest that the local flare is possibly an immune mechanism with features of a reactive-like arthritis with complement activation and production of inflammatory cytokines without ignoring other potentially distinctive mechanisms. To our knowledge, this is the first description of the possible local involvement of the complement pathway in a synovial fluid and tissue immune reaction to hylan G-F 20.

Methods, results and discussion

We present the case of a 55-year-old woman with bilateral OA, who had undergone left knee meniscectomy 9 years previously and received in the last year two 5 ml treatments with hylan G-F 20 in both knees at our hospital. The radiograph diagnosed a severe grade 4 OA in the left knee without evidence of chondrocalcinosis in either knee.

Approximately 12 h after the third injection in both knees the patient presented with local massive swelling accompanied by severe pain limited to the left knee joint. Systemic reactions were not described. Decompression by ultrasound-guided aspiration of the left knee joint was performed in the next 12 h yielding 65 ml of yellowish synovial fluid filled with inflammatory cells (WBC 33,950/μm³, RBC 1500/mm³, neutrophils 60%, eosinophils 18%, monocytes 19% and lymphocytes 3%). The swelling and pain returned rapidly to the previous state and an emergency arthroscopic irrigation and debridement were performed the following day. Synovial fluid and tissue were collected for further processing. The blood reports indicated acute phase reactants with slightly elevated ESR at 33 mm/h (Westergren method, normal values = 0–27 mm/h) and high C-reactive protein (CRP) at 6.4 mg/dl (normal values range from 0 to 1 mg/dl). Microscopic examination of freshly prepared synovial fluid for crystal deposits, Gram’s stain, and cultures of the synovial fluid were all negative. The treatment included oral ciprofloxacin 500 mg twice daily. The patient presented with local massive swelling accompanied by severe pain limited to the left knee joint. Systemic reactions were not described. Decompression by ultrasound-guided aspiration of the left knee joint was performed in the next 12 h yielding 65 ml of yellowish synovial fluid filled with inflammatory cells (WBC 33,950/μm³, RBC 1500/mm³, neutrophils 60%, eosinophils 18%, monocytes 19% and lymphocytes 3%). The swelling and pain returned rapidly to the previous state and an emergency arthroscopic irrigation and debridement were performed the following day. Synovial fluid and tissue were collected for further processing. The blood reports indicated acute phase reactants with slightly elevated ESR at 33 mm/h (Westergren method, normal values = 0–27 mm/h) and high C-reactive protein (CRP) at 6.4 mg/dl (normal values range from 0 to 1 mg/dl). Microscopic examination of freshly prepared synovial fluid for crystal deposits, Gram’s stain, and cultures of the synovial fluid were all negative. The treatment included oral ciprofloxacin 500 mg twice daily.

Enzyme-linked immunosorbent assays (ELISAs) for markers of complement activation and cytokines were performed. The concentrations of interleukin-1 beta (IL-1β), C5a and C5b-9 in synovial fluid were determined using commercially available ELISAs (IL-1β and C5b-9: BD Biosciences, Franklin Lakes, NJ; C5a: R&D Systems, Minneapolis, MN), according to the manufacturers’ instructions. Our experimental results demonstrate that the concentrations of IL-1β in the case patient (210 pg/ml) were nine times higher than in RA patients (25 pg/ml) and 100 times higher than in OA patients (2 pg/ml) [Fig. 2A(i)]. The complement fragment C5a concentration was 9 ng/ml, closer to the average concentration of 10 ng/ml in the synovial fluids of the three OA patients, but almost four times higher than the average concentration of 2.5 ng/ml in the synovial fluids of the three OA patients [Fig. 2A(ii)]. The concentration of the C5b-9 complex was highest in our case patient at 5500 ng/ml, compared to the average concentrations in synovial fluids of 4500 ng/ml in the RA patients and 800 ng/ml in the OA patients [Fig. 2A(iii)]. Therefore, we hypothesize that the local immune profile changed in the patient from a low inflammatory response characteristic of OA to a highly inflammatory arthritis pattern with a predominant synovial T lymphocytic infiltrate [Fig. 1(C(i))], alongside a neutrophilic and eosinophilic infiltration in the synovial exudate around hylan G-F 20 particles [Fig. 1(B(ii) and (iii))]. This immunological response is interpreted in a large part to be secondary to the activation of the complement pathway, since the lymphocytic infiltrate observed in OA is usually perivascular and of mixed B and T cell pattern, with scattered presence of mast cells. The numerous macrophagic component is considered secondary to OA and also to the hylan deposits [Fig. 1(B(iv) and C(iv))].
The presence of C5b-9 deposits in synovial tissue correlates well with the presence and extent of inflammatory synovitis in OA and RA. In the case patient, we observed diffuse presence of stained C5b-9 deposits intermixed with inflammatory cells in the fibrinous exudate and vascular endothelial cells with a membrane-like pattern. RA patients showed a similar distribution, whereas in OA patients there were not any deposits. Three marker genes with major roles in local joint inflammation, IL-1β, IL-6, and a neutrophil marker myeloperoxidase (MPO), were also analyzed by quantitative real-time polymerase chain reaction (RT-PCR) in synovial tissues of the case patient alongside the three OA and three RA patients. The presence of numerous neutrophils in the inflamed knee joint may contribute greatly in our case patient to local flare, and the production of abundant cytokines such as IL-1β and IL-6 and specific enzymes such as MPO. IL-1β and IL-6 are major inflammatory cytokines expressed in synovium in RA, but at lower levels in OA patients. In our patient synovium, IL-1β mRNA was 34-fold higher compared to OA patient samples and 15-fold higher than in synovium from RA patients. In addition, the IL-6 mRNA levels were similar to those for IL-1β and 36-fold and 16-fold higher than those in the OA and RA patients respectively. MPO gene expression was five times higher in the patient injected with hyaluronan G-F 20 than in the RA and OA patients; however, the patients in the RA and OA groups demonstrated little elevation. This might be a direct result of the high number of neutrophils present in the synovium of the case patient and a less severe OA phenotype because MPO is considered a possible marker of early OA.

Fig. 1. A. Inflammatory cells (macrophages) with hyaluronan G-F 20. (i, ii) Mixed cell population isolated from fibrinous exudate and synovial tissue showing macrophages containing small vesicles with hyaluronan G-F 20 at 50× (i) and 200× (ii) magnification. B. Histologic photomicrographs of synovial tissue of an OA patient treated with hyaluronan G-F 20. (i–iv) Sections of left knee synovium stained with H&E. Scale bars represent 200 um. (i) A particle of hyaluronan G-F 20 in fibrinous exudate is surrounded by a thick cuff of inflammatory cells (100× magnification); (ii) the infiltrate is composed of neutrophils and eosinophils (400× magnification); (iii) the inflammatory infiltrate is predominant in the fibrinous exudate with relative sparing of the synovial membrane (100× magnification); and (iv) an old particle of hyaluronan G-F 20 in the synovium is associated with a palisading macrophagic reaction and new acute inflammatory infiltrate (200× magnification). C. Photomicrographs of immune cells in synovial tissue from hyaluronan G-F 20-treated OA patient. (i–iv) The distribution of inflammatory cells is shown in a representative area of the synovial membrane. (i) T lymphocytes, CD3; (ii) B lymphocytes, CD20; (iii) mast cells, CD117; and (iv) macrophages, CD68. All images were captured at 200× magnification. The presence of positive cells was counted by ImageJ as percentage vs total number of nuclei of cells counterstained with toluidine blue. We identified numerous T lymphocytes and macrophages within the inflammatory areas surrounding hyaluronan G-F 20 particles. D. Histologic photomicrographs of cancellous bone and marrow of an OA patient treated with hyaluronan G-F 20. (i–ii) Sections of cancellous bone and marrow stained with H&E. (i) Particles of hyaluronan G-F 20 in bone marrow surrounded by macrophagic reaction and inflammatory cells (40× magnification). Hylan G-F 20 presence is indicated by the letter H and acute inflammatory infiltrate by the letter I; letter M demonstrates the macrophagic reaction. C. Photomicrographs of immune cells in synovial tissue from hyaluronan G-F 20-treated OA patient. (i–iv) The distribution of inflammatory cells is shown in a representative area of the synovial membrane. (i) T lymphocytes, CD3; (ii) B lymphocytes, CD20; (iii) mast cells, CD117; and (iv) macrophages, CD68. All images were captured at 200× magnification. The presence of positive cells was counted by ImageJ as percentage vs total number of nuclei of cells counterstained with toluidine blue. We identified numerous T lymphocytes and macrophages within the inflammatory areas surrounding hyaluronan G-F 20 particles. D. Histologic photomicrographs of cancellous bone and marrow of an OA patient treated with hyaluronan G-F 20. (i–ii) Sections of cancellous bone and marrow stained with H&E. (i) Particles of hyaluronan G-F 20 in bone marrow surrounded by macrophagic reaction and inflammatory cells (40× magnification). Hylan G-F 20 presence is indicated by the letter H and trabecular bone by the letter T; (ii) expanded image of squared box in (i) with inflammatory infiltrate composed of numerous lymphocytes and eosinophils on the left side and macrophages with a giant cell on the right side (400× magnification).
There are several possibilities explaining the immunological profile of the case patient. First, the reaction indicated similarities with flares in crystal-induced arthropathies associated with cell surface cleavage of C5 and formation of the complement attack complex (C5b-9)\textsuperscript{14} while the phagocytosis of hylan G-F 20 by macrophages triggered the NLRP3 inflammasome activation with release of inflammatory mediators and influx of neutrophils in the joint\textsuperscript{18}. Second, an alternative hypothesis of previous sensitization\textsuperscript{5} via deposits of hylan G-F 20 is worthy of consideration. We have recently observed a case of total knee replacement in a female patient also affected by grade 4 OA. In addition of numerous eosinophils and scattered neutrophils [Fig. 1(D (i) and (ii))], deposits of hylan with palisading macrophages and giant cells and scattered lymphocytes were equally present in the bone marrow and synovium. However, to make conclusions about a comparable process, more studies of the acute flare reaction in similar patients are necessary. Third, in this study we have presented similarities with inflammatory arthropathies such as reactive arthritis and RA illustrated by the presence of numerous T lymphocytes and macrophages, complement activation, and release of specific pro-inflammatory cytokines. Nevertheless, this association might not be ideal when the cellular composition of the immune infiltrate, almost virtual absence of CD20\textsuperscript{+} and CD38\textsuperscript{+} cells and the presence of eosinophils, is carefully examined.

While severe, the inflammatory knee changes were only temporarily present. Benefiting from rehabilitation therapy, by 6 months, the knee functionality was fully recovered, and at 1 year, the same performance level was observed as before the acute reaction occurred.

In this study we cannot assess why the flare did not occur in the contralateral joint or at other times during the treatment regimen. The severity of OA, the prolonged presence of the foreign substance in the synovium and the accumulation exceeding a certain threshold may be contributing factors to a local sensitization in certain susceptible individuals. Additional studies of the acute flare reaction in patients with multiple treatments of viscosupplementation are warranted.

**Authors’ contribution**

The authors alone are responsible for the content and writing of the paper.

**Conflict of interest**

The authors have no competing interests.

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