Sequential synovial fluid sampling suggests plasma and synovial fluid IL-6 vary independently in rheumatoid arthritis

Sir, Inflammatory cytokines, including interleukin-6 (IL-6), are raised in rheumatoid arthritis (RA). Single paired daytime samples show that the IL-6 and TNF-α concentrations in synovial fluid exceed blood concentrations during the day [1–3], and these and other data suggest that joints are the source. Recent data have shown that plasma IL-6 in RA follows a circadian pattern with a peak at 6 a.m. [4]. If IL-6 is generated inside joints and diffuses into plasma, it might be expected that the synovial fluid IL-6 concentration would also undergo a large diurnal variation. Such investigations necessitate the development of a safe and acceptable synovial catheter allowing sequential synovial fluid sampling. Following the approval of the United Bristol Healthcare Trust Research Ethics Committee, we undertook a daytime pilot study for this purpose. In addition we obtained preliminary data for IL-6 variations in paired synovial fluid and plasma samples.

We recruited six volunteers aged 36–64 yr with RA according to the criteria of the American College of Rheumatology. Each volunteer had active disease (at least three swollen and three tender joints) and a large knee effusion. After establishing the technique, we obtained paired blood and synovial fluid samples in three patients. One patient was taking prednisolone 7.5 mg daily, but no other patient was taking glucocorticoids and none had had intra-articular or intra-muscular glucocorticoids for more than 10 weeks. After we had obtained written consent, each patient was admitted and remained on bed rest between 8.30 a.m. and 5.30 p.m. With the patient on the bed, we used an aseptic non-touch technique including sterile gown and gloves to insert a synovial catheter. The knee was exposed, a large area of skin carefully cleaned with iodine 1% antiseptic solution, and sterile drapes were used to produce an aseptic field. Up to 2 ml bupivacaine 0.5% was infiltrated under the skin adjacent to the lateral parapatellar joint space and towards the synovial membrane. After local anaesthesia was obtained, a sterile disposable intravenous (i.v.) cannula (14 gauge BD Venflon; Becton Dickinson Infusion Therapy, Sweden) was inserted into the parapatellar joint space. The cannula was connected to a three-way tap with flexible extension (BD Connecta Plus 3; Becton Dickinson Infusion Therapy). Sterile film wound dressings (C-View; Unomedical, UK) enabled the synovial cannula to remain perpendicular to the skin. After taking microbiological and orthopaedic advice, we chose not to use prophylactic antibiotics. A standard i.v. cannula was used for sequential blood sampling. No samples were taken for at least 1 h after cannulae insertion. The i.v. cannula was flushed with 10 ml 0.9% saline after insertion and before and after each blood sample. The first 2 ml of each sample taken from the knee and the first 4 ml of each sample taken from the i.v. cannula were discarded. Paired blood and synovial fluid samples were taken through the day. Plasma and synovial fluid samples were stored at −80°C and analysed using solid-phase sandwich ELISA (enzyme-linked immunosorbent assay) kits (Diaclone Research, detection limit 2 pg/ml, interfer- and intra-assay variation < 6%). A colleague not directly involved in the project asked each volunteer for comments about their experience.

The synovial catheter allowed sequential synovial fluid sampling over 8 h. From the independent interviews, the procedure was acceptable to volunteers. The only adverse effect noted was slight knee stiffness in two volunteers, and there were no other adverse effects. In addition we obtained a small number of paired synovial fluid and blood samples (Fig. 1). Plasma IL-6 decreased exponentially by 95%, from a mean [95% confidence interval (CI)] of 19.1 (34.1, 4.1) pg/ml at 10 a.m. to <2 (<2), <2) pg/ml at 5 p.m. (paired t-test, P < 0.05). These results are similar to previously published data [4]. Synovial fluid IL-6 changed little during the 8 h (non-significant decrease of 30%), with 10 a.m. IL-6 mean (95% CI) 12.0 (17.5, 6.5) ng/ml and 5 p.m. mean 7.9 (11.1, 4.7) ng/ml. The patient with the highest synovial fluid IL-6 concentration had the lowest plasma IL-6 concentration.

Developing a safe and acceptable synovial catheter was the prime reason for this study. We found that sequential synovial fluid samples can be taken safely through a synovial catheter over 8 h by a technique that is acceptable to volunteers and avoids repeated arthrocentesis. Our very preliminary values are similar to previous...
A CCR-5 antagonist inhibits the development of adjuvant arthritis in rats

Sir, Chemokine receptor CCR5 is preferentially expressed on TH1 lymphocytes and has been reported to have important roles in the pathogenesis of rheumatoid arthritis (RA). Yang et al. [1] demonstrated that a CCR5 antagonist inhibits the development of arthritis in the collagen-induced arthritis (CIA) model in mice. They showed that the inhibition of the development of arthritis is not caused by affecting the generation of collagen-sensitized T cells but by interfering with their migration to joint lesions. Recently, Vierboom et al. [2] reported the interesting finding that a CCR5 antagonist inhibited the development of CIA in rhesus monkeys. They showed that systemic administration of a small molecular weight antagonist of CCR5, SCH-X, suppressed the development of CIA in a monkey model of rheumatoid arthritis (RA).

Rheumatoid arthritis is a chronic, destructive, inflammatory, polyarticular joint disease, characterized by massive synovial proliferation and subintimal infiltration of inflammatory cells, followed by the destruction of cartilage and bone. In the pathogenesis of RA, inflammatory cytokines such as IL-6 and TNF-α play important roles and this chronic inflammation results in cellular damage of affected joints [3, 4]. Chemokines are involved in the process of leucocyte transmigration into sites of inflammation. CCR5 and CXCR3 are expressed on TH1 cells, and CCR4 and CXCR3 on TH2 cells [5, 6]. Evidence shows that RA is a TH1-dominant disorder and that CCR5- and/or CXCR3-expressing cells are enriched in affected joints of RA patients [7, 8]. We now provide evidence showing that systemic administration of TAK-779, a non-peptide compound with a small molecular weight (Mr 531.13) [9], inhibits the development of adjuvant-induced arthritis (AIA) in rats.

Seven-week-old female Lewis rats were obtained from Charles River Japan (Yokohama, Japan). Complete Freund’s adjuvant (CFA) was prepared by suspending heat-killed Mycobacterium butyricum (Difco Laboratories, Detroit, MI, USA) in liquid paraffin at 10 mg/ml. CFA-induced arthritis was stimulated by injection of 100 μl of the CFA emulsion intradermally at the base of the left paw. TAK-779 was obtained from Takeda (Osuka, Japan). Treatment commenced at the onset of the disease;

single time-point data [1, 3, 4], but in addition they suggest that there is not a straightforward relationship between synovial fluid and plasma IL-6. A variation was reported in blood IL-6 from normal volunteers with sleep deprivation, but our blood variation is four times greater than the maximum seen in these normal volunteers [5]. Although this is a small pilot study with large confidence intervals and one volunteer was taking a low dose of glucocorticoid, the results suggest that plasma IL-6 remains relatively unchanged. This challenges the notion that plasma IL-6 is merely a reflection of synovial IL-6 production. The true relationship between synovial fluid and plasma levels needs further investigation, in particular in relation to night-time variation. Such studies should be possible using a synovial catheter.

FIG. 1. IL-6 concentrations (mean and 95% confidence interval) in paired synovial fluid (SF) and plasma samples from three volunteers with rheumatoid arthritis (time points for synovial fluid are displaced by 10 min for clarity).

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