Letters to the Editor

Transforming growth factor-β1, interleukin-1β and collagenase activity in subchondral bone of the femur and the severity of osteoarthritis of the hip

Sirs,

Factors and mechanisms determining the development of osteoarthritis are not yet defined. Apart from interleukins or metalloproteinases, growth factors such as transforming growth factor-β (TGF-β) or bone morphogenetic proteins (BMPs) are often listed as compounds which can be involved in the pathogenesis of osteoarthritis (1).

The activation of repair processes are responsible for increased osteosynthesis and production of osteophytes. In the literature available there is little data assessing the content of growth factors that could participate directly in the development of osteoarthritis of the hip joint.

The aim of the study was to determine the relationship between the content of TGF-β1, IL-1β and the activity of collagenase in subchondral bone of the femoral head and the severity of osteoarthritic changes of the hip joint.

32 samples of subchondral bone were collected from patients with hip osteoarthritis during total hip replacement. There were 21 women and 11 men enrolled. The mean age was 66 (range from 37 to 80 yrs.).

For the evaluation of the severity of osteoarthritis of the hip, the Kellgren and Lawrence classification was used (2). It is a five-degree scale classifying osteoarthritric changes on the basis of the hip X-ray in AP view. Along with an development of these changes, a joint is placed into a higher class. Ten joints (31.2%) were classified as group 2 according to Kellgren-Lawrence classification, 11 as group 3 (34.4%) and 11 as group 4 (34.4%).

The content of total protein in bone samples was measured with the use of BCA-Protein Assay Reagent (Pierce, Beijerland, Holland) (3). The concentration of TGF-β1 and IL-1β was determined with the use of the enzyme-linked immunoassay (ELISA) Quantikine Human Immunoassay Test (R & D Systems, Minneapolis, USA) (4, 5). Collagenase activity was assayed fluorimetrically (Bachem, Biochemica GmbH Heidelberg, Germany) (6).

For quantity data, the variability range, dispersions and means, as well as normal distribution errors, have been marked. Results cited in this paper involve the data-cleansing procedure. Correlation coefficients between the quantities analysed were measured. The data from the analysed subgroups were compared with the use of the t-Student test. The data was statistically significant at the level below 0.05.

We found direct proportional correlation between the content of TGF-β1 converted into total protein and the severity of osteoarthritic changes ($p < 0.05$) (Fig. 1). The mean content of TGF-β1 in patients with minimal osteoarthitic changes of the hip joint classified to group II according to Kellgren-Lawrence classification was 0.86 pg/μg. In patients with moderate hip osteoarthritis (group III) the mean content of TGF-β1 was 1.24 pg/μg. The highest mean content of TGF-β1 (1.72 pg/μg) was observed in patients with the most severe osteoarthritic changes (classified as group IV). Similar correlation but with no statistical significance was observed for IL-1β. We found no correlation between the activity of collagenase and the severity of osteoarthritis.

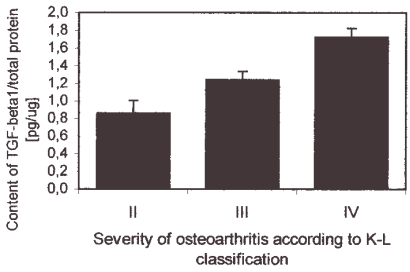

Fig. 1.

Our results confirm the vital role of TGF-β1 in the pathogenesis of bone formation in osteoarthritis of the hip joint. In patients with more severe osteoarthritic changes of the hip joints, the content of TGF-β1 in subchondral bone layer was higher. This observation is also confirmed in animal studies by Scharstuhl *et al*. (7). In experimentally induced knee osteoarthritic changes in mice, adding proteins such as mLAP-1, SMAD6 and SMAD7 as intracellular TGF-β1 and BMP inhibitors, caused a decrease in osteophyte formation in the joint. The role of TGF-β1 in osteophyte formation during osteoarthritis in the experimental model was also postulated by Van der Berg (8).

Further examination of the role of TGF-β1 and other cytokineses in the development of osteoarthritis can lead to their application in treatment or prevention of this particular illness.

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Successful treatment of necrotizing vasculitic lesions after infusion of iloprost in a patient with cryoglobulinemia and chronic HCV infection

Sirs,

We report a case of a patient with cryoglobulinaemia and chronic HCV infection who presented necrotizing vasculitic lesions treated with iloprost who positively responded to this treatment. Cryoglobulinemic vasculitis (CV) is an immune-complex-mediated systemic vasculitis involving small-medium sized vessels, in which a causative role of HCV in 80-90% of patients has been definitely established. Purpura and rheumatic complaints are the most common symptoms found in HCV associated CV (1). HCV genomic sequences cannot be integrated into the host genome, since the HCV is a single-stranded RNA virus without a DNA intermediate, not directly cytophatic,(2) but it can trigger the immunological alterations indirectly by exerting a chronic stimulus to the immune system. New insights on the pathogenetic mechanisms of HCV-related immunological disorders include the evidence that the HCV envelop protein E2, interacting with CD81 molecule expressed on B-lymphocytes, may increase the frequency of VDJ rearrangement in antigen-reactive B-cell. One possible consequence may be the activation of anti-apoptotic Bcl-2 protoncogene that leads to extended B-cell survival. The B-lymphocyte expansion is responsible for a

wide autoantibody and immune-complex production, including mixed cryoglobulins (3).

Our patient developed psoriatic lesions at the age of 32, and after 8 years migrant arthralgias. At the age of 51 he developed necrotic papular lesions and asthenia, and after 6 months a diagnosis of HCV infection was made, on the evidence of anti-HCV antibodies positivity. On admission to our department, physical examination revealed numerous erythematous papular nodular lesions and some ulcero-necrotic lesions, livaedo reticularis, a purplish mottling of the skin, and multiple psoriatic lesions. Laboratory findings showed ESR 75 mm/hr, AST 169 IU/l, ALT 147 IU/l; the presence of antibody anti-HCV was positive, detected by RIBA test. HCV-RNA test was positive, showing an active viral replication. Latex-agglutination test for rheumatoid factor was strongly positive. Serum immunoelectrophoresis revealed type III cryoglobulinaemia.

Because of the worsening of psoriatic lesions with the use of interferon alpha and the contraindication to the use of immunosuppressors and prednisone due to the active viral replication, we chose to use iloprost on the basis of the following considerations. Iloprost is a stable prostacyclin analogue, successfully used to treat Raynaud's phenomenon and digital ulcers associated with connective tissue diseases (4). The best known mechanisms of the drug action are vasodilation and antiplatelet effect, which have been demonstrated, may be involved in the exertion of its clinical efficacy but apart from these above-mentioned mechanisms, lymphocyte adhesion and IL-1 stimulated expression of ICAM-1 and ELAM-1 also exhibit a significant reduction in the presence of iloprost; moreover, this drug induces fibrinolysis, increasing in red cell deformability, inhibition of vascular smooth muscle cell proliferation, down-regulation of leukocyte adhesion molecules and the inhibition of lymphocyte production of proinflammatory cytokines, such as TNF-α, IL-1 and IL-6 (5). It has been furthermore demonstrated that prostacyclin or its stable analogue partially inhibits the adhesion of polymorphonuclear cells and lymphocytes to endothelial cells, and this has to be considered in the treatment of ischemic diseases whatever the aetiology is, since it is well-established that neutrophil adhesion to the endothelium plays a central role in ischemia-reperfusion injury (6). We therefore used i.v. iloprost 2 ng/kg/min, administered for 6 hours daily for 5 days. At the end of the treatment, a substantial improvement occurred (Fig. 1) with the disappearance of necrotizing lesions.

We conclude that the immune response to HCV and the cryoglobulins related to infection can lead to several clinical features, among which papular-nodular and ulcero-necrotic must be considered. In this case, but also in other kinds of coetaneous involvement, iloprost may be a useful tool when other therapies are not available, or as an adjunct to steroids and immunosuppressors.

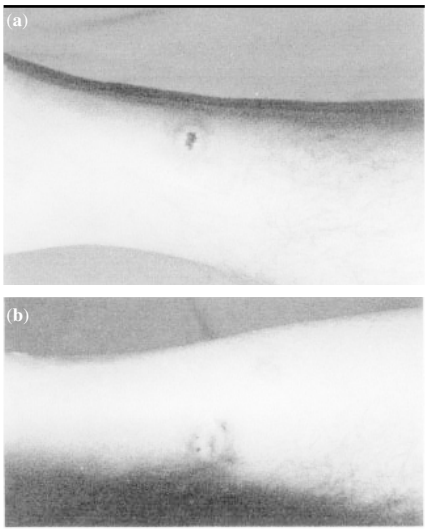

Fig. 1. Vasculitic lesions before (**a**) and after (**b**) treatment with iloprost.

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Reprints will not be available from the author.

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A mistake in the history of aspirin

Sirs,

We would like to point out a widespread misapprehension in the rheumatological literature. In several histories of aspirin (1-4) two Italian chemists, Luigi Valentino Brugnatelli and Felice Fontana, are credited with the extraction of salicin from willow bark in 1826, two years earlier than Johann Andreas Buchner, Professor of Pharmacy in Munich, Germany (5). Brugnatelli and Fontana were eminent Italian chemists at the beginning of the 19th century: Brugnatelli was Professor of Chemistry at the University of Pavia and Fontana was the first Director of the Museum of Natural History in Florence, but neither of them conducted research on salicylic compounds. Furthermore, they both had passed away before 1826: Felice Fontana died in 1805 and Brugnatelli in 1818.

The first extraction of the active component of willow bark was actually performed in 1824, i.e. two years before the reported date, by two other Italian researchers, both of whom were pharmacists – Bartolomeo Rigatelli in Verona, and Francesco Fontana in Lazise, near Verona. Rigatelli named the drug "salino amarissimo antifebbrile" (bitter febrifugal saline), while Fontana utilized the same term – salicin – that a few years later was to be adopted by Buchner. Unlike Brugnatelli and Felice Fontana, they were not connected with academic world and they published the results of their studies in local journals (6, 7). This may explain the *quiproquo*, together with the assonance between Brugnatelli and Rigatelli and the homonymy between Felice and Francesco Fontana.

It was not easy to retrace the origin of the mistake. The earliest paper in the international literature in which we found a citation of Brugnatelli and Fontana was that by Julius Wohlgemuth in 1899 (8), but they had already been erroneously cited in 1845 by Raffaele Piria (9), another Italian chemist who in 1838 (10) was the first to isolate salicylic acid from salicin. Progess in any field is built of of many little stones and we are aware of the relatively trivial nature of this detail. Nevertheless, we think that is worthwhile to re-establish the truth and to recognize the merits.

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