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Role of cytokines in gonarthrosis and knee prosthesis aseptic loosening

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Abstract Cytokines, which have been demonstrated in synovial fluids during various joint diseases, play an important role in mediating synovial inflammation and in regulating the immune response of many inflammatory processes. We studied synovial fluid, serum, and synovial fragments obtained from 33 patients—10 affected by serious gonarthrosis requiring a prosthetic implant, 8 with knee prosthesis aseptic loosening, and (as controls) 15 affected by degenerative meniscopathies—to evaluate the degree of inflammation and level of interleukins (IL-2, IL-4, IL-6, IL-10) and interferon γ secretion. Histological analysis revealed slightly more infiltration by inflammatory cells in the synovial tissue of patients with gonarthrosis and knee prosthesis aseptic loosening than in that of the control group, with a high prevalence of macrophages. Moreover, we observed enhanced production of the studied cytokines, especially in synovial fluid as compared to serum, indicating that in the pathological conditions examined the inflammatory events are mainly localized. Because the role of these cytokines is to modulate inflammation, better knowledge of the involvement of cells and their soluble mediators in articular damage could guide immunomodulating treatment.

Key words Cytokines · Immune response · Synovial inflammation · Joint diseases

Introduction

Cytokines play an important role in mediating synovial inflammation and regulating the immune response of many joint diseases. The presence of multiple cytokines has been demonstrated in synovial fluid specimens from patients with various arthropathies.[23] The sources of these cytokines are polymorphonuclear leukocytes, macrophages, lymphocytes, endothelial cells, and synovial tissue.[2,21] These cells and their mediators interact in regulating the immune response.

Interleukin-2 (IL-2) is, above all, a growth factor for T lymphocytes; and its activity increases in the presence of other factors, such as IL-4, whose activity is inhibited by interferon-γ (IFNγ). Moreover, IL-4 down-regulates monocyte production of IL-1, IL-6, and tumor necrosis factor-α (TNFα) and can therefore exert an antiinflammatory force. For this reason it is an important mediator in regulation of the immune system.

Abnormalities in IL-2 production and in the expression of its receptors have been found in various pathological situations such as multiple sclerosis, rheumatoid arthritis, systemic lupus erythematosus, acquired immune deficiency syndrome (AIDS), and Hodgkin's lymphoma.[1,24] It seems that IL-6 plays an important role in the course of joint inflammation. This molecule can be synthesized by various cells, such as monocytes, fibroblasts, and endothelial cells; but it is also produced by macrophages, T and B lymphocytes, granulocytes, osteoblasts, and mast cells.[8] Some studies have demonstrated an increased concentration in the synovial fluid of patients with rheumatoid arthritis but not in those with osteoarthritis.[6,18] Overproduction of IL-6 is common in rheumatoid arthritis, multiple myeloma, cirrhosis, and Castleman's syndrome.[9,16,22] IL-6 probably also plays a pathogenetic role in chronic polyarthritis, as suggested by finding increased levels in synovial fluid. Some cytokines down-regulate the production of other soluble mediators. Among them, IL-10 can inhibit IFNγ production,[14] and the release of IL-1, IL-6, and TNFα by macrophages; it can also reduce the expression of antigen major histocompatibility complex II (MHCII) on the monocyte surface and therefore the antigen-presenting cell (APC) function of macrophages.[7] Macrophages have an important role in inducing and maintaining articular inflammation, bone resorption, and induction of prosthetic aseptic loosening. For this reason the cytokines that regulate their functions are

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extremely important. IFNγ stimulates reactive oxygen intermediate production by macrophages[5] and is involved in bone growth; it also inhibits resorption, perhaps by partially reducing osteoclast production.[11] Because of these features, IFN-γ is useful for treating chronic polyarthritis[27]: It modulates the function of macrophages and so reduces joint pain.

The aim of our study was to examine synovium-infiltrating cells and cytokine production in various osteoarticular diseases. We wanted to evaluate their role in immunoinflammation and evaluate the possible control of the inflammatory process by pharmacological modulation of their production.

Patients and methods

Patients and samples

After giving informed consent, 33 patients entered this study: 10 (3 men, 7 women; mean age 70 years) affected by serious gonarthrosis requiring a prosthetic implant (GPI group) and a homogeneous group of 8 (5 men, 3 women; mean age 70 years) with knee prosthesis aseptic loosening (KPL group). The prosthesis we used was the GKS butterfly-rotating hinged (Permedica, Merate, Italy) knee prosthesis (tricompartmental gliding knee prosthesis stabilized on all planes). The interval between primary and revision surgery was 7.2 years. As controls we studied 15 subjects (10 men, 5 women; mean age 42 years) affected by degenerative meniscopathies undergoing selective arthroscopic surgical meniscectomy. Synovial fluid (SF), serum, and synovial fragments were obtained from all patients to evaluate the degree of inflammation and level of cytokine secretion.

Synovial fluid samples were aspirated into sterile heparinized containers and stored at −80°C until analyzed. Serum samples were aliquoted after centrifugation of whole peripheral blood at 3000 rpm for 10 min and the aliquots stored at −80°C until use. Synoviectomy specimens were obtained from the GPI and KPL patients during surgery and from the control group during knee arthroscopy. The synovial fragments were fixed in formalin and embedded in paraffin for histological analysis; sections were cut, stained with hematoxylin and eosin, and scored independently by two observers according to the degree of cellular infiltration and the number of blood vessels.

A score ranging from 0 to 4 was used to classify the values obtained as follows. (1) The number of blood vessels: 0, ≤3; 1, 4–9; 2, 10–15; 3, 16–21; 4, ≥22. (2) PMNs: 0, <3; 1, 3–10; 2, 11–22; 3, 23–85; 4, >85. (3) Lymphocytes: 0, 0–50; 1, 51–200; 2, 201–400; 3, 401–600; 4, >600. (4) Plasma cells: 0, <3; 1, 3–25; 2, 26–85; 3, 86–150; 4, >150. The inflammation score was obtained by summing the parameters.[13] The mean values for total infiltration and for each component were obtained for each group of patients.

Cytokine assay

The concentrations of IL-2, IL-4, IL-6, IL-10, and IFNγ in synovial fluid and serum were determined by an enzyme-linked immunoassay (Genzyme, Cambridge, MA, USA), performed according to the manufacturer's instructions.

Statistical analysis

The Snedecor-Fisher F-test for variance analysis was used. $P = 0.01$ was considered statistically significant.

Results

Histological analysis revealed slightly more infiltration by inflammatory cells in the synovial tissue of patients with GPI and KPL than in the control group. The mean score for infiltration by macrophages was higher than that for the other cells in all groups, especially in KPL patients (Table 1). Lymphocytes were more prevalent in the synovial tissue of GPI patients than in the other two groups (Table 1), whereas only sporadic plasma cells were present, with no substantial differences among the groups. The cytokine concentrations in the synovial fluid and serum are summarized in Tables 2 and 3, respectively.

Interferon-γ was detectable in the serum of all the patients, with higher mean values for the control patients (9.5 pg/ml) than for the KPL patients (8.7 pg/ml) or the GPI patients (3.7 pg/ml) (Fig. 1). In the synovial fluid IFNγ was present at higher concentrations in the control group (12.6 pg/ml) than in the KPL and GPI groups (7.0 and 5.7 pg/ml, respectively) (Fig. 1) but the differences were not significant.

Interleukin-10 was found in only a small percentage of the patients' sera: 2 of 8 KPL patients (mean

Table 1. Mean scores for infiltration in synovium of patients affected by joint diseases

Site	GPI	KPL	Control group
Blood vessels	3.93 ± 0.95	2.86 ± 1.20	2.81 ± 1.25
Lymphocytes	1.85 ± 0.68	1.14 ± 0.37	1.18 ± 0.40
Macrophages	2.4 ± 0.9	3.06 ± 0.5	2.3 ± 1.0
Plasma cells	0.75 ± 0.44	0.85 ± 0.37	0.90 ± 0.30
Score	8.3 ± 1.8	8.4 ± 1.5	7.1 ± 2.3

Results are means ± SD
GPI, gonarthrosis requiring a prosthetic implant; KPL, knee prosthesis loosening

Table 2. Concentrations of cytokines in joint fluid

	GPI		KPL		Control group	
Cytokine	Mean	Range	Mean	Range	Mean	Range
IFNγ	5.7	1.7–13.0	7.0	4–10	12.6	1.6–55.0
IL-10	30.0	1–69	35.0	21–52	18.3	0.9–50.0
IL-6	81.5	0.9–318.0	900.0	461–2194	51.7	1.9–112.0
IL-4	533.85	137–1056	180.0	128–220	214.0	61–784
IL-2	61.8	15–112	67.5	58–83	98.1	69–212

Results are expressed as picograms per milliliter
IFNγ, interferon-γ; IL, interleukin

Table 3. Concentrations of cytokines in serum

	GPI		KPL		Control group	
Cytokine	Mean	Range	Mean	Range	Mean	Range
IFNγ	3.7	1–14	8.7	3–17	9.5	1.2–28.0
IL-10	27.0	20–34	14.0	11–17	15.8	12–20
IL-6	0.3	0–1	0.6	0–2	0	0
IL-4	57.8	15–158	23.0	13–30	165.5	30–717
IL-2	1.4	1.1–1.8	0	0	1.5	0.9–2.0

Results are expressed as picograms per milliliter

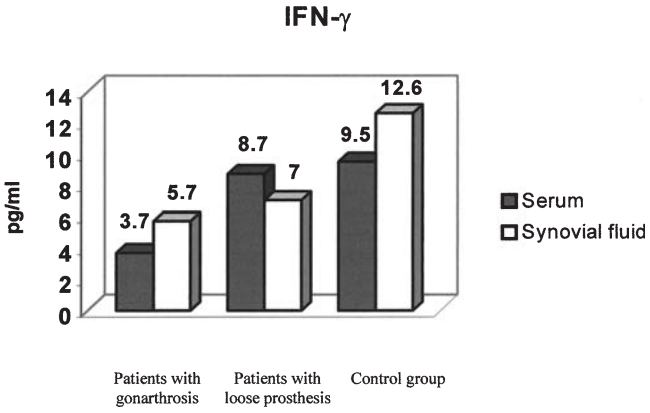

Fig. 1. Interferon-γ (*IFN-γ*) mean values in serum and synovial fluid of the three groups

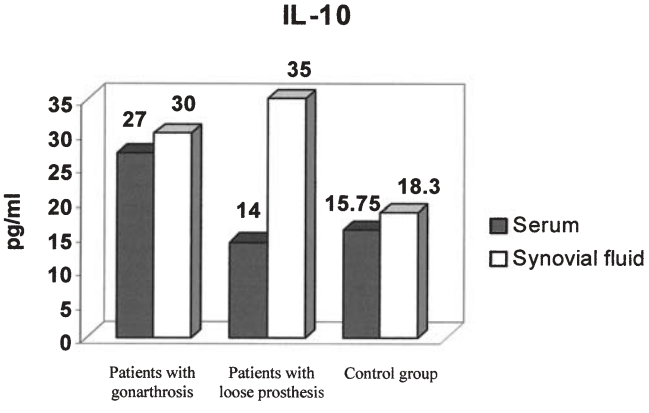

Fig. 2. Interleukin-10 (*IL-10*) mean values in serum and synovial fluid of the three groups

14 pg/ml), 2 of 10 GPI patients (mean 27 pg/ml), and 4 of 15 controls (mean 15.8 pg/ml). In contrast, the IL-10 level in synovial fluid was always detectable, with mean values of 35 pg/ml in KPL patients, 30 pg/ml in GPI patients, and 18.3 pg/ml in the controls (Fig. 2).

Interleukin-6 was almost undetectable in the sera of all patients (Fig. 3), whereas in the synovial fluid of the KPL group we found high levels of this cytokine (900 pg/ml). Even though high concentrations were detectable in the other two groups (81.5 pg/ml in the GPI group and 51.7 pg/ml in the control group) (Fig. 3), the differences from the KPL group were significant (P = 0.01).

Serum IL-4 levels were higher in the controls (165.5 pg/ml) than in the GPI (57.8 pg/ml) or KPL (23 pg/ml) patients (Fig. 4). In contrast, in synovial fluid we found the highest IL-4 levels in the GPI group (P = 0.01) [533.85 (PGI) vs 214 (controls) vs 180 pg/ml (KPL)] (Fig. 4).

Interleukin-2 was undetectable in the sera of the KPL patients and in only a few patients in the other two groups (Fig. 5). Like the other cytokines IL-2 was always present in the synovial fluid in all three groups [61.8 (GPI) vs 67.5 (KPL) vs 98.1 pg/ml (controls)] (Fig. 5) with significant amount (P = 0.01) in the control group.

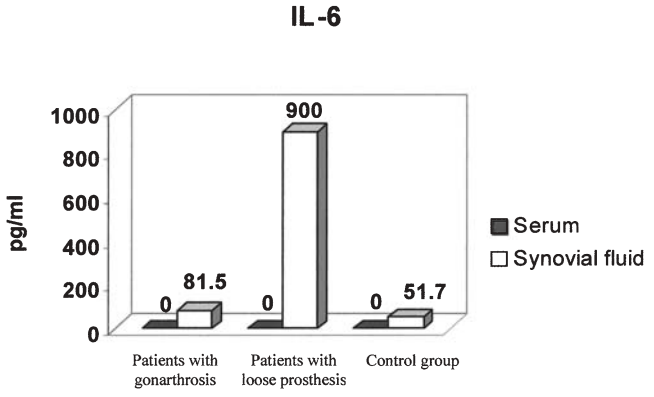

Fig. 3. Interleukin-6 (*IL-6*) mean values in serum and synovial fluid of the three groups

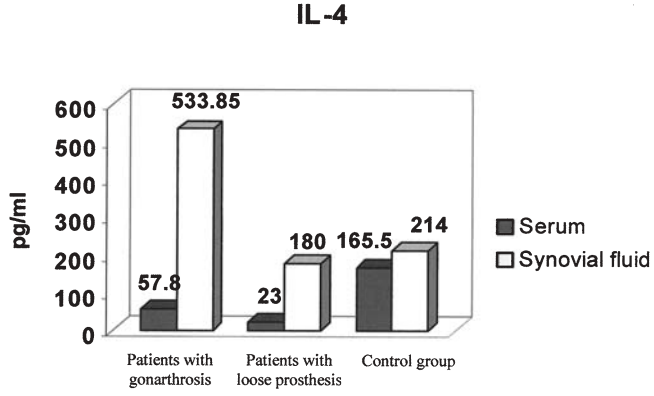

Fig. 4. Interleukin-4 (*IL-4*) mean values in serum and synovial fluid of the three groups

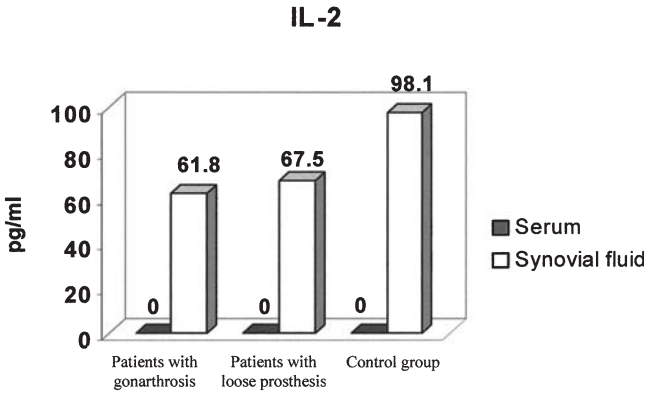

Fig. 5. Interleukin-2 (*IL-2*) mean values in serum and synovial fluid of the three groups

Discussion

In various diseases that involve synovial tissue, it is possible to detect an increase in proinflammatory or antiinflammatory cytokines (or both) in synovial fluid.[23,25] These mediators may be responsible for progression or inhibition of inflammation and bone resorption or inhibition of bone formation.[10] We observed enhanced production of cytokines, especially in synovial fluid compared to serum, indicating that in the pathological conditions examined the inflammatory events are mainly localized.

All of the pathological entities we studied are characterized by chronic inflammation of the synovium, but we observed markedly high cellular infiltration in the synovial tissue of the KPL patients, with a high prevalence of macrophages. Moreover, we found significantly high levels of IL-6, a cytokine mostly produced by macrophages, in the synovial fluid of these patients, confirming previous findings that suggested a pathogenetic role for IL-6 in KPL.[15] However, compared to the other cytokines (IFNγ and IL-10), IL-6 was present at high levels in the synovial fluid in the other two groups as well, emphasizing its pro-inflammatory role.

Interleukin-10 levels were similar in the synovial fluid of the KPL and GPI groups, whereas they were lower in the control group. The role of this cytokine is to modulate inflammation; in fact, it may inhibit IL-6 production by macrophages.[20] The lower level of IL-10 with respect to IL-6 suggests that IL-10 production was insufficient to inhibit the inflammatory progression induced by IL-6. IL-4 also down-regulates IL-6 synthesis by macrophages.[4] In our study IL-4 was increased in the synovial fluid in all groups, particularly the GPI patients, and its levels were inversely correlated with those of IL-6. In fact, the IL-4 concentration was higher than that of IL-6 in the GPI and control groups, whereas it was lower in the KPL patients. This further substantiates the possibility of reciprocal modulation by these cytokines.

The low levels of IFNγ found mostly in the serum and synovial fluid of KPL and GPI patients are compatible with the presence of important inflammation mechanisms and rearrangement in these groups. It seems, in fact, that IFNγ can exert an antiinflammatory action and inhibit bone resorption processes.[11,17]

Interleukin-2, usually produced by T lymphocytes, was increased in synovial fluid of all groups. This molecule has an important role in inflammatory progression and activation of cytotoxic lymphocytes[12]; in particular, it is an important autocrine growth factor for T lymphocytes. For this reason, detection of this cytokine in the synovial fluid in all groups, and particularly the control group, suggests that lymphocytes also contribute to the progression of inflammation. Even if lymphocytes were not the main infiltrating cells, it is known that they are present also in synovial fluid.[13] Previous studies demonstrated that T cells in the syno-

vial fluid of patients with an arthropathy express a T helper-1 (Th1) phenotype; in fact, they are able to produce mainly IL-2 and IFNγ.[19] Our results show a concordance in the synovial fluid levels of these cytokines. Their concentrations were higher (statistically significant) in the control group than in the other groups; nevertheless, we observed a rise in IL-4, the cytokine produced by Th2 lymphocytes.[19] This seems to be in contrast with the other studies. However it is known that mastcells also can produce IL-4,[28] and these cells represent an important inflammatory cell population in the synovium.[3,29] Even if we did not study these cells, it is possible that they play an important role in the diseases we studied. Because various inflammatory cells may synthesize cytokines that have a reciprocal role in regulating their own production, it is difficult to establish a precise role for each in the course of immunoinflammation and to determine whether a cytokine has an immunoregulating function rather than a pathogenetic one.

The heterogeneous cytokine levels observed in patients affected by the same disease suggest various therapeutic approaches, depending on the severity of the inflammation, and afford a guide to prognosis. For example, knee prosthesis aseptic loosening may be suspected in patients with increased IL-6 in the synovial fluid, especially in association with low INFγ levels. Moreover, in patients affected by arthrosis the levels of proinflammatory cytokines could indicate the rate of disease progression and suggest targeted antiinflammatory treatment. The complex mechanisms that regulate bone-remodeling cells and their interactions with immune cells are only partly understood. Nevertheless, the IFNγ-mediated suppression of osteoclastogenesis is well known, and some[26] have suggested therapeutic use of this cytokine in inflammation-induced tissue breakdown. Because the IFNγ concentration in synovial fluid of patients with aseptic loosening was at low levels in our study, we suggest that IFNγ could represent a novel therapeutic approach to treating these diseases. Further studies are required to better define the involvement of cells and soluble mediators in articular damage and, above all, to guide immunomodulating treatment.

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