Production of interleukin 8 by cultured synovial cells in response to interleukin 1 and tumor necrosis factor

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Summary. Both interleukin 1α (IL-1α) and tumor necrosis factor α (TNFα) stimulated the production of interleukin 8 (IL-8) by synovial cells in time and dose dependent manners. Enhanced chemotactic activity of polymorphonuclear cells (PMN) in culture supernatants of synovial cells was neutralized with anti-IL-8 antibody, thus showing synovial cells to be capable of secreting IL-8 which may contribute to PMN accumulation in rheumatoid inflamed joints.

Key words: Interleukin 8 – Synovial cells – Rheumatoid arthritis

Introduction

The accumulation of polymorphonuclear cells (PMN) in synovial fluid is characteristic of rheumatoid arthritis (RA). Several factors including C5a, LTB4, platelet activating factor and platelet factor 4 have been found and confirmed as chemotactic and activating factors for PMN in the synovial fluid of RA patients [1]. Activated PMN cause the release of lysosomal proteinases, oxygen radicals and arachidonic acid metabolites which directly bring about tissue damage and inflammation in RA. Recently, interleukin 8 (IL-8)/neutrophil attracting peptide 1 (NAP-1), secreted by activated monocytes as well as by various types of cells, has been identified as a major neutrophil chemotactic factor [2]. The amount of IL-8 has also been shown to be increased in synovial fluid from RA patients [3]. We conducted this study to determine whether synovial cells are capable of producing IL-8 in response to inflammatory cytokines such as interleukin 1 (IL-1) and tumor necrosis factor (TNF).

Materials and methods

Reagents. Human recombinant interleukin 1α (2 x 10^7 U/mg thymocyte co-mitogen assay [4]), tumor necrosis factor α (2 x 10^6 U/mg

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tigate whether polyclonal antibodies for cytokines would be able to abolish IL-1 and TNF-induced IL-8 production.

The effects of metabolic inhibitors on IL-8 production by synovial cells were also investigated. Synovial cells were pretreated with Actinomycin D (5 x 10^{-7} M) or cycloheximide (5 x 10^{-6} M) for 4 h [9, 10]. They were then rinsed three times with phosphate buffered saline and incubated with medium containing IL-1α (100 pg/ml) or TNFα (1 ng/ml). After 24 h incubation at 37°C, the amount of IL-8 in each culture supernatant was determined by RIA to assess the effects of metabolic inhibitors. The viability of the treated cells was measured by a trypan blue dye exclusion test and found not to have changed, compared to that of control cells.

Results

Induction of IL-8 production in synovial cells by IL-1 and TNF

Figure 1 illustrates the effects of carrier-free human recombinant IL-1α (100 pg/ml) and TNFα (1 ng/ml) on IL-8 production in synovial cells. Both IL-1 and TNF increased this production in a time-dependent manner. IL-8 production was also found to increase after 6 h of stimulation and to continue to do so progressively thereafter.

IL-1α and TNFα were applied to synovial cells at different doses for 24 h. Figure 2 shows the amounts of immunoreactive IL-8 in the culture supernatants. Both IL-1α and TNFα increased IL-8 production in proportion to their concentrations. IL-1α was more capable of stimulating the IL-8 synthesis by synovial cells than TNFα. At 100 pg/ml, IL-1α and TNFα induced a 72-fold and a 25-fold enhancement of IL-8 production, respectively. Human recombinant IL-1β (Otsuka Pharmaceutical Co. Ltd., Tokushima, Japan) essentially showed the same activity as that of IL-1α (data not shown). Contamination of lipopolysaccharide (LPS) in the cytokines used in this study was less than 0.05 ng/mg protein. To confirm further that the effects of cytokines were not due to contaminated LPS, experiments were conducted using polymyxin B (10 μg/ml) or boiled cytokines. The addition of polymyxin B caused no change in IL-8 production induced by IL-1α or TNFα (data not shown). IL-1α (100 pg/ml) and TNFα (1 ng/ml), after being boiled in water for 10 min, reduced IL-8 production to 2.8% and 4.2% that of the control respectively. Pretreatment of IL-1 and TNF with their antibodies also decreased the effect to 7.5% and 10% of the control, respectively. It is thus evident that IL-1 and TNF were capable of stimulating IL-8 production by synovial cells.

Chemotactic activity of culture supernatants. The chemotactic activity in the culture supernatants of synovial cells was measured to determine whether immunoreactive IL-8 induced by IL-1 would exhibit biological activity. Pretreatment of these cells with IL-1α (100 pg/ml) for 8 h significantly increased chemotactic activity toward PMN in the culture supernatant (Table 1). Pretreatment with TNF had a similar effect (data not shown). The neutralizing antibody specific for IL-8 completely abolished this activity.

Fig. 1. Kinetics of IL-8 production from synovial cells by IL-1α or TNFα. Synovial cells at confluency in 48 well culture dishes were incubated with medium alone (→), human recombinant IL-1α 100 pg/ml (→) or human recombinant TNFα 1 ng/ml (→) for the periods indicated. The amounts of immunoreactive IL-8 in the culture supernatants were determined by RIA. The data represent mean values and SD of duplicate measurements

Effects of metabolic inhibitors on IL-8 induction. To determine whether newly synthesized protein and RNA are required for the induction of IL-8 by IL-1 and TNF, the effects of metabolic inhibitors on induction were analyzed. Pretreatment of synovial cells with cycloheximide (5 x 10^{-6} M) or actinomycin D (5 x 10^{-7} M) for 4 h decreased cytokine-induced IL-8 production (Table 2). Pretreatment with cycloheximide and actinomycin D for 4 h inhibited approximately 80% of net protein synthesis and RNA synthesis of cells respectively (data not shown). These metabolic inhibitors did not reduce cell viability. Thus, IL-1 and TNF-induced IL-8 production by synovial cells required both protein and RNA synthesis.