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The combined effects of anti-TNFα antibody and IL-1 receptor antagonist in human rheumatoid arthritis synovial membrane

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Abstract

TNFα and IL-1 are the pivotal cytokines involved in rheumatoid arthritis (RA). More recently, the biological therapy targeting TNFα or IL-1 has been impressively effective for many RA patients, however, it remains insufficient in some patients. In the present study, we examined the combined effects of two agents against TNFα and IL-1 in human RA synovial membrane. Synovial explants (an ex vivo model) and synovial fibroblasts (an in vitro model) were prepared from 11 RA patients, and then anti-TNFα antibody (Anti-TNFα) and IL-1 receptor antagonist (IL-1Ra), either alone or in combination, were added to the synovial explants and fibroblasts. IL-6 and MMP-3 production were measured after incubation. As a result, their production significantly decreased by the combination of agents compared with the control group in both the synovial explants and fibroblasts. The efficacy of this combination was also observed for IL-6 production compared with each agent alone in the synovial explants, and for IL-6 and MMP-3 production compared with each agent alone in the synovial fibroblasts. Therefore, the combination of Anti-TNFα and IL-1Ra appears more beneficial in synovial membrane, particularly when compared with a single agent alone.

Keywords: Rheumatoid arthritis; Synovial membrane; Anti-TNFα antibody; IL-1 receptor antagonist; Combination

1. Introduction

Rheumatoid arthritis (RA) is an autoimmune disease that causes inflammation and destruction of the joints. Although its etiology remains uncertain, various cytokines are known to play key roles as mediators that induce abnormality [1]. In particular, tumor necrosis factor α (TNFα) and interleukin 1 (IL-1) are located the upstream of the cytokine cascade, and are pivotal mediators in the induction of an excessive reaction in vivo [2]. Although TNFα and IL-1 have many similar biologic effects, mainly, TNFα leads to systemic and local inflammation, whereas IL-1 is involved in cartilage and bone destruction locally [3].

Recently, treatments that target TNFα and IL-1 have begun to be applied in the clinical setting. However, although these therapies can obtain mostly excellent results, they are ineffective in some patients [4,5]. The reason for these poor outcomes is unclear. Some possibilities are that the therapeutic agents do not adequately infiltrate the local tissue, such as joints, to suppress the progression of joint destruction, a cytokine other than the target cytokine is the cause of these disadvantage events, and more than one cytokine is involved in them.

In previous studies, inhibition of both TNFα and IL-1 simultaneously in animal models or tissue systems has resulted in good outcomes [6–11]. These results expect the efficacy of the combined therapy of TNFα and IL-1 when the improvement is not at all or insufficient by the single agent therapy.

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In the present report, we measured IL-6 production as an inflammation mediator and MMP-3 production as a marker of cartilage destruction, and investigated the effects of single and combination therapy of anti-TNFα antibody (Anti-TNFα) and IL-1 receptor antagonist (IL-1Ra) (competitively inhibits binding of IL-1) using RA patients' synovial explants and cultured synovial fibroblasts.

2. Materials and methods

2.1. Reagents

TypeIcollagenase, Dulbecco's modified Eagle's medium (DMEM), fetal bovine serum (FBS), and penicillin–streptomycin were purchased from Gibco (Grand Island, NY). Monoclonal anti-human-TNFα/TNFSF1A antibody (Anti-TNFα), recombinant human IL-1Ra, recombinant human TNFα (TNFα) and recombinant human IL-1β (IL-1β) were purchased from R&D Systems (Minneapolis, MN).

2.2. Synovial explants

Synovial membrane was obtained from 11 RA patients (all woman; mean age, 59.6 years; age range, 36–84 years) who met the revised criteria of the American College of Rheumatology [12] and who were undergoing surgical treatment (nine total knee joint replacements, one total hip joint replacement, and one extensor digitorum muscle rupture tendon suture). Informed consent was obtained from all patients. Surgical tissue samples were washed three times in PBS, cut roughly and divided into sets for the synovial explant and the cultured synovial fibroblast experiments.

The synovial explants were prepared as previously described by Ounissi-Benkalha et al. [13]. Briefly, the fragmenting synovial membrane was dissected from underlying fibrous and fatty tissue and incubated for 24 h at 37 °C with DMEM containing antibiotics. Synovial explant (100 mg) was mixed with 1 ml DMEM in each well of a 24-well plate. The wells were then divided into four groups: (1) the control group, nothing added; (2) the group receiving Anti-TNFα alone; (3) the group receiving IL-1Ra alone; and (4) the combined group receiving both Anti-TNFα and IL-1Ra.

According to the neutralization of human TNFα bioactivity information supplied with R&D Systems, which is the agent we used, 0.5 μg/ml Anti-TNFα was assumed to neutralize 1 ng/ml TNFα completely. On the other hand, IL-1Ra is required at a 10- to 100-fold or greater amount than IL-1 for activity, and 1000-fold the amount is required to completely control IL-1 [14]. Each of the four groups was treated with the appropriate amount of agent and then cultured for 24 h at 37 °C with 5% CO_2. Culture supernatant was then preserved at −80 °C.

2.3. Synovial fibroblasts

The synovial fibroblasts were prepared as previously described [15]. Briefly, the synovial tissue fragments were minced, incubated with 1 mg/ml TypeIcollagenase in serum-free DMEM for 3 h at 37 °C, centrifuged at 1000 rpm for 5 min, then, non-adherent cells were removed, and washed four times with DMEM. These cells were cultured in DMEM supplemented with 10% FBS, 100 U/ml penicillin, and 100 μg/ml streptomycin at 37 °C with 5% CO_2. We changed the supernatant once every 2–3 days, and when confluence was reached in 7–14 days, performed passage using 0.25% trypsin. The synovial fibroblasts used for this experiment were at passage 3–8, at which time they were a homogeneous population of fibroblasts. The synovial fibroblasts were plated at a density of 10^5 in 1 ml DMEM per well in a 24-well plate, and the wells were then randomly divided as follows: (1) the control group, nothing added; (2) the group receiving Anti-TNFα alone at low dose (0.2 μg/ml), moderate dose (1.0 μg/ml) or high dose (5 μg/ml); (3) the group receiving IL-1Ra alone at low dose (20 ng/ml), moderate dose (100 ng/ml) or high dose (500 ng/ml); and (4) the combined group receiving Anti-TNFα and IL-1Ra in triplicate with Anti-TNFα 0.2 μg/ml + IL-1Ra 20 ng/ml, Anti-TNFα 1 μg/ml + IL-1Ra 100 ng/ml, and Anti-TNFα 5 μg/ml + IL-1Ra 500 ng/ml. After incubating for 1 h at room temperature, TNFα 10 ng/ml and IL-1β 0.5 ng/ml were added to all wells including the control group. The wells were then cultured for 24 h at 37 °C with 5% CO_2, and the supernatant gathered and stored at −80 °C.

2.4. IL-6 and MMP-3 determination

IL-6 and MMP-3 in the supernatant were determined using the Endogen® Human Interleukin-6 ELISA and Quantikine® Human MMP-3 (total) Immunoassay, respectively.

2.5. Statistical analysis

For the synovial explant and synovial fibroblast experiments, each of IL-6 and MMP-3 production in the control group was defined as 100%, and the production with both the single and two agents together is shown as the percentage compared with the control group. The statistical significance of differences between groups was assessed by analysis of ANOVA (Tukey–Kramer test). Correlation coefficients were obtained by Pearson's correlation coefficient analysis. A p value less than 0.05 was considered significant for all experiments.

3. Results

3.1. Combined effects of Anti-TNFα and IL-1Ra in human RA synovial explants (ex vivo model)

To inhibit both TNFα and IL-1 appeared to be effective as both these cytokines cause unusual reactions and these two have somewhat different actions in vivo. Therefore, the combined effect of Anti-TNFα and IL-1Ra was examined using the human RA synovial explants (Figs. 1 and 2).

At first, we measured the TNFα level in the synovial explants control group supernatant, it was a mean of 400 pg/ml (range