Original Article
Effect of human recombinant interleukin-10 on cytokines in adjuvant arthritis rat

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Abstract: Rheumatoid Arthritis (RA) is a kind of chronic systemic autoimmune disease mainly presented as arthropathy. Its clinical manifestation includes progressive and symmetry joint destruction, even joint deformity and loss of function. Rat adjuvant arthritis (AA) model is a commonly used animal model to investigate human RA mechanism. This research applied human recombinant Interleukin-10 (hIL-10) to treat AA rats to explore hIL-10 therapeutic effect. A total of 30 Wistar rats were used to establish AA rat model by complete Freund’s adjuvant subcutaneous injection and treated with hIL-10. Rat weight was measured. Hind limb joint swelling degree was tested by drainage method. Serums IL-1, IL-4, and TNF-α levels were detected by ELISA. Rat weight in AA model group was obviously lower than the normal control. After modeling, the rats appeared significant hind limb joint swell. After hIL-10 treatment for 4 weeks, arthrocele degree in treatment group markedly reduced. Serology detection revealed that serum IL-1 and TNF-α level decreased, whereas IL-4 content elevated after hIL-10 treatment (P < 0.05). hIL-10 can alleviate AA induced weight loss and arthrocele, and can impact IL-1, IL-4, and TNF-α secretion. It showed therapeutic effect on AA.

Keywords: Adjuvant arthritis, hIL-10, inflammatory cytokine

Introduction

Rheumatoid Arthritis (RA) is a chronic systemic autoimmune disease that mainly causes systemic small arthropathy [1]. Its clinical feature is progressive and symmetric joint destruction, then causing joint swelling, stiffness, deformity, or dysfunction [2]. Epidemiological investigation found that the incidence of RA was about 0.5% in our country, mainly in 20 to 50 years old [3]. Since RA lesions involve cartilage and bone tissue, it often causes joint deformity and dysfunction, leading to disability or loss of labor. It further causes serious physical and mental burden and pressure, and also brings serious burden for the society [4]. The pathogenesis of RA is a complex biological process that is unclear, current treatment stays in treating inflammation and sequelae. Non-steroidal anti-inflammatory drugs (NSAIDs) are commonly used for RA treatment. It plays an anti-inflammatory effect to relieve symptoms. However, it cannot alleviate illness, and may cause side effects, such as gastrointestinal symptoms and kidney damage [7]. Although corticosteroids can anti-inflammation, it also cannot prevent joint destruction or block the disease development. Furthermore, long-term usage may lead to adverse reactions such as osteoporosis and obesity [8]. With the development of science and technology, multiple cytokines were detected in RA patients and were found to participate in RA process through complex regulation network [9]. Interleukin-10 (IL-10) is a kind of single glycoprotein composed of 178 amino acids that mainly secreted by TH2 cells [10]. Study showed that IL-10 is associated with a variety of autoimmune diseases [11], such as systemic lupus erythematosus, rheumatoid arthritis, psoriasis, etc. [12, 13]. To further investigate the effect of human recombinant IL-10 (hIL-10) on AA, we established AA rat model by complete Freund’s
hIL-10 in treating AA

adjuvant and observed weight and joint change. We also used hIL-10 to treat AA rat to detect inflammatory cytokines changes and explore the therapeutic effect of hIL-10 on AA rat.

Materials and methods

Main reagents and instruments

Complete Freund’s adjuvant (Sigma, USA); recombinant human IL-10 (Peprotech, USA); 3% pentobarbital sodium (Guangzhou organic chemical reagent factory, China); Rats IL-1 kit (Neobioscience, Shanghai); Rats IL-4 kit (Neobioscience, Shanghai); Rat TNF-α kit (Neobioscience, Shanghai); Microplate reader (Biotek, USA); Thermostatic water bath (Beijing Changyuan experiment equipment factory, China), etc.

Experimental animal

A total of 30 male Wistar rats weighted 180 g were bought from Binzhou Medical University. The rats were fed in thermostatic chamber (25±2°C) with relative humidity at 50±5% and 12 h day and night. The rats received free eating and drinking.

Rats were used for all experiments, and all procedures were approved by the Animal Ethics Committee of Binzhou Medical University.

AA rat model establishment

The Wistar rats were randomly equally divided into five groups, including normal control, model group, hIL-10 high, middle, and low dose group. The rats in model group and hIL-10 group received 0.8 ml complete Freund’s adjuvant solution left plantar subcutaneous one-off injection. The rat’s mental state, activities, food intake, joints condition, and weighing were observed daily. AA rats were randomly divided into four groups after 2 weeks, including model group, hIL-10 high, middle, and low dose group. The rats in hIL-10 group received hIL-10 intraperitoneal injection every other day for continuous 4 weeks at 2 μg·Kg⁻¹, 10 μg·Kg⁻¹, and 50 μg·Kg⁻¹, respectively. The rats in model group and normal control received equal amount of sterile normal saline at the same way.

Arthrocele degree detection

Arthrocele degree was measured by drainage method. The posterior limb was put into the graduated transparent container full of water. Overflow water volume after ankle of posterior limb immersed to determine swelling degree. The swelling degree was tested at modeling, 1 week after modeling, hIL-10 treatment, and 1, 2, 3, 4 weeks after treatment. The rat weight was also measured at the same time. The measurement was repeated for three times and the mean value was calculated as swelling degree index.

ELISA

Rat blood was collected for 1 ml from inner canthus using capillary glass tube at before modeling, modeling, and 2 and 4 weeks after treatment to test serum IL-1, IL-4, and TNF-α levels. The serum was centrifuged at 12000 g and 4°C for 5 min. 50 μl standard substance or sample was added to each well with three replicates at 37°C for 30 min. After removing the fluid, the plate was washed by buffer for 3 times and added 50 μl enzyme-labelled reagent for at 37°C for 30 min. After washed by buffer for 3 times, the plate was added with color developing agent A and B at 37°C for 15 min. Then 50 μl stop buffer was added to each well and the plate was read at 450 nm to calculate concentration based on standard curve.

Statistical analysis

SPSS 20.0 was applied for data analysis. All data were presented as mean ± standard deviation and tested by t test or ANOVA. P < 0.05 was considered as statistical significant.

Results

hIL-10 impact on AA rat weight

The average weight of rats at the beginning was 183.20 ± 23.24 g. No statistical difference was
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observed among five groups (P > 0.05). The weight showed elevation in different degree with time extension (Figure 1). At the end of experiment, the mean weight of rats were 318.53±21.71 g, 247.26±23.37 g, 261.78±27.18 g, 274.27±22.06 g, and 295.35±24.62 g in normal control, model group, hIL-10 low, middle, and high dose group, respectively. As shown in Figure 1, the rats weight in model group and treatment group declined compared with normal control (P < 0.05), while it increased in treatment group compared with model group (P < 0.05).

hIL-10 impact on AA rat arthrocele

Drainage method was applied to measure rat posterior limb arthrocele during experiment (Table 1). No significant difference was observed among each group at the beginning (P > 0.05). Then the swelling degree presented changes in different degree with time extension. The rats in model group and hIL-10 treatment group showed obviously arthrocele compared with normal control (P < 0.05). Compared with AA model group, the posterior limb swelling degree in hIL-10 treatment group presented markedly alleviation with dose-dependent (P < 0.05).

hIL-10 impact on AA rat inflammatory cytokines expression

Serum inflammatory cytokines IL-1, IL-4, and TNF-α levels were detected at starting, modeling, and 2 and 4 weeks after treatment (Figure 2). No statistical difference was observed among each group at the beginning (P > 0.05). Then the swelling degree presented changes in different degree with time extension. The rats in model group and hIL-10 treatment group showed obviously arthrocele compared with normal control (P < 0.05). Compared with AA model group, the posterior limb swelling degree in hIL-10 treatment group presented markedly alleviation with dose-dependent (P < 0.05).

Table 1. Joint swelling degree changes (ml)

<table>
<thead>
<tr>
<th>Group</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6 (week)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>1.18±0.06</td>
<td>1.21±0.08</td>
<td>1.25±0.09</td>
<td>1.28±0.11</td>
<td>1.32±0.11</td>
<td>1.34±0.08</td>
<td>1.36±0.09</td>
</tr>
<tr>
<td>Model group</td>
<td>1.16±0.07</td>
<td>1.83±0.09</td>
<td>2.85±0.11</td>
<td>3.54±0.13</td>
<td>3.52±0.12</td>
<td>3.75±0.16</td>
<td>3.26±0.10</td>
</tr>
<tr>
<td>hIL-10 low dose</td>
<td>1.15±0.05</td>
<td>1.75±0.10</td>
<td>2.89±0.12</td>
<td>3.37±0.11</td>
<td>3.26±0.16</td>
<td>3.11±0.10</td>
<td>2.94±0.13</td>
</tr>
<tr>
<td>hIL-10 middle dose</td>
<td>1.16±0.07</td>
<td>1.81±0.09</td>
<td>2.75±0.13</td>
<td>3.33±0.15</td>
<td>3.17±0.14</td>
<td>3.02±0.11</td>
<td>2.91±0.12</td>
</tr>
<tr>
<td>hIL-10 high dose</td>
<td>1.18±0.08</td>
<td>1.83±0.11</td>
<td>2.83±0.17</td>
<td>3.28±0.15</td>
<td>3.11±0.13</td>
<td>2.97±0.18</td>
<td>2.88±0.11</td>
</tr>
</tbody>
</table>

hIL-10 low, middle, and high dose was 2 μg·Kg⁻¹, 10 μg·Kg⁻¹, and 50 μg·Kg⁻¹, respectively. *P < 0.05, compared with normal control; #P < 0.05, compared with model group.

Figure 2. Serum IL-1, IL-4, and TNF-α expression. *P < 0.05, compared with normal control; #P < 0.05, compared with model group (A: IL-1 expression; B: IL-4 expression; C: TNF-α expression).
Serum IL-1 and TNF-α level elevated (Figure 2A and 2C), while IL-4 content reduced (Figure 2B) after modeling (P < 0.05). After hIL-10 treatment for 2 and 4 weeks, serum IL-1 and TNF-α level declined (Figure 2A and 2C), whereas IL-4 content enhanced (Figure 2B) compared with model group (P < 0.05).

Discussion

RA is a kind of chronic autoimmune disease that mainly damage synovial membrane, cartilage, and bone tissue. It is a type of symmetric and progressive arthritis occurred in small joints [14]. It was reported that RA pathogenesis often accompanied with abnormal IL-1, IL-4, and TNF-α expression. IL-1, also known as lymphocytes stimulating factor, is mainly produced by activated macrophages. It can stimulate mesenchymal cells in bone tissue secreting a large number of collagenase and prostaglandins, destroying bone collagen and affecting bone absorption, resulting in cartilage and synovial tissue lesions [15]. IL-4 is a kind of anti-inflammatory factor generated by activated T cells. It plays a role to inhibit inflammation by participating in humoral immune reaction through promoting TH2 cells reaction and affecting T cells and B cells proliferation and differentiation through restraining inflammatory cytokines secretion [16]. TNF-α is mainly produced and secreted by macrophages. It stimulates inflammation by activating cell degranulation and peroxidase secretion to enhance the phagocytosis of neutrophils and enhancing MCH class I antigen and IL-1 expression to promote neutrophil adhesion to epithelial cells [17]. Early research suggested that AA rat model was an ideal animal model for human RA pathogenesis investigation. HSP65 in the mycobacterium tuberculosis in complete Freund’s adjuvant shows high similarity with the molecular structure of autoantigen HSP60 in rat articular cartilage. Both of these two proteins can be recognized by T cells, thus inducing autoimmune reaction which gives priority to joint damage [18, 19]. Therefore, this study explored hIL-10 impact on cytokines expression in AA rat and its therapeutic effect on AA.

Our results showed that the rat’s weight in model group and treatment group declined compared with normal control, while it increased after four weeks’ hIL-10 treatment compared with model group. It suggested that hIL-10 affected rat weight increase. Drainage detection revealed that compared with AA model group, the posterior limb swelling degree in hIL-10 treatment group presented markedly alleviation. Previous report indicated that human RA pathogenesis accompanied with abnormal IL-1, IL-4, and TNF-α expression. Moreover, it was found that IL-1 and TNF-α can promote inflammation progress as inflammatory cytokines, whereas IL-4 can inhibit inflammation as belonging to anti-inflammatory cytokine [20]. Further study demonstrated that compared with AA model group, serum IL-1 and TNF-α level declined, whereas IL-4 content enhanced in hIL-10 treatment group (P < 0.05). It suggested that hIL-10 can affect AA occurrence by inhibiting IL-1 and TNF-α expression and upregulating IL-4 level.

In conclusion, hIL-10 can promote weight gain and alleviate arthrocele in AA model. It showed therapeutic effect on AA by affecting IL-1, IL-4, and TNF-α secretion.

Disclosure of conflict of interest

None.

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References


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