Rheumatoid arthritis is a chronic systemic autoimmune disease characterized by damage to the joints. This damage is caused by chronic inflammation of synovial joints, degeneration, and the formation of pannus blood vessels, and it affects arthrodial cartilage, subcartilaginous osseous lamella, ligaments, and tendons. This is followed by destruction of cartilage, bone and joint capsules, leading to joint deformation and functional incapacitation. Qubi Zhentong Recipe (祛痹镇痛方, QZR) can activate blood and resolve stasis, expel wind, and relieve pain, which has a good therapeutic effect in the clinic. In this study, we applied gene chip technology to determine whether QZR affects the gene expression profile of collagen-induced arthritis (CIA), which could further explain the mechanism.

METHODS

Experimental Animals

Sixty healthy male Wistar rats, weighing 110 ± 10 g, SPF level, were provided by Animal Lab Center, Zhejiang Chinese Medical University with certification No. SYXXK (Zhe) 2003-0003.

Drugs

QZR includes Scorpio 9 g, Eupolyphagaisensis Walker 10 g, Caharsiis molossus Linnaeus 6 g, Scolopendra subspinipes 5 g, Semen Persiace 10 g, Flos Carthami 6 g, Boswellia carteri 6 g, Achyranthes bidentata 10 g, and Arystolochia mollissima H 10 g. The medicine was mixed with water at a ratio of 1:7, and boil for 20 min after 2 h dip in water. The medicine was filtrated, and then the decoctions were put together. The decoction was concentrated to
1 g/mL with a water bath kettle. The certificate number is 20101112, as provided by the Zhejiang Traditional Chinese Medicine and Western Medicine Integration Hospital Manufacturing Laboratory.

**Experimental Materials**

The Illumine gene chip (Setrix RatRef-12 Expression BeadChip, USA) was used, which contains 31,000 probes, and represents 28,000 rat genes. Type II bovine collagen (BC II, lot number: 2002-1) and incomplete Freund's adjuvant (IFA, lot number: 7002) were from Chondrex Inc, USA. Glacial acetic acid was from Hangzhou Chemical Reagent Ltd., China (lot number: 20100924). The toe volume measuring instrument was from Shangdong Medical Scientific Equipment Factory, China. The GeneChip scanner (Affymetrix, USA) 3000 7G 4C was used.

**Animal Model Establishment**

Under aseptic conditions, 0.1 mol/L glacial acetic acid was applied to completely dissolve BC II by a water bath kettle. The concentration of BC II was 4 mg/mL. BC II was then placed into a refrigerator at 4 °C overnight and then mixed isometrically with IFA, shaken, and homogenized. The resultant BC II 2 mg/mL emulsion was kept at 4 °C in a refrigerator. The model was based on the references. The 0.5 mL BC II emulsion was injected into the tail of model rats intracutaneously.

**Modeling Standards**

Every healthy rat's left hind limb toe cubage was measured as basic cubage before the rats became inflamed. The arthritis index (AI) was graded every week. The model was estimated by the AI 18 days after the first injection. The AI grade scale is as follows: grade 0, no redness and swollen; grade 1, the toe joint is mildly swollen, with some inflammation of a single claw or food pad; grade 2, mild redness and swollen joints, with more than two claws or foot pads, or ankle joint inflammation; grade 3, medium-level redness of the joints and mild dysfunction; and grade 4, severe redness and swollen joints, with rigidity even deformity and serious dysfunction. The standard model was successfully established if the AI greater than 4, and the swelling volume on left hind limb below ankle was over 6 mL.

**Groups and Medication**

We randomly chose 10 rats from 60 rats as the control group, and the other 50 rats were used for the CIA model. Twenty rats were randomly chosen from 34 successful CIA models and assigned into two groups: the model group (n=10) and the QZR group (n=10). The CIA model group was given normal saline 22.9 g/(kg·d) intragastrically. The QZR group was fed intragastrically with the Chinese medicine decoction at a dose of 22.9 g/(kg·d) (10 times the clinical adult equivalent dose). Both groups were administered once a day, for 30 days.

**Tissue Collection**

The rats were anesthetized by 10% chloral hydrate with abdominal cavity injection for sacrifice. Rats were fixed in the overhead position, and sterilized by iodine and alcohol. A longitudinal incision was made from the middle of the knee joint until a 3 cm × 3 cm area in the center of the knee joint was exposed. We observed a smooth, bright, light yellow synovium, which extended downward from the patella. We performed blunt dissection of the synovial and fiber layers by ophthalmologic tweezers. Synovial tissue was peeled off completely, and then the margin part was clamped gently with ophthalmologic tweezers. Using a sterilized scalpel, the synovial tissue was dissected, which weighed approximately 7–10 mg. Samples were placed into a freezes tube and then placed in liquid nitrogen.

**State of Swelling of the Toe**

A toe volume measurement machine was used to observe the cubage of the left hind limb toe of rats. The area used for observation was below the bare skin. The area (mL) and days of observation was recorded, which included day 18 (when the model was made), 33, and 48.

**Synovial Gene Expression Profile Testing of CIA Rats**

Recovery and Determination of Total RNA

A total of 5 mg of tissue sample was ground into small pieces in liquid nitrogen. Then 1 mL of Trizol was added while the tissue still grinding. The tissue was then brought to room temperature. Samples were placed into 1.5 mL EP tubes and 200 µL of chloroform was added. The mixture was shaken for 20 s, and then placed under room temperature for 2–15 min. Samples