Histamine H4 Receptor Expression in Human Synovial Cells Obtained from Patients Suffering from Rheumatoid Arthritis

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While histamine H4 receptor (H4R) has been implicated in immune system disturbances in different organ tissues, the presence and possible roles of H4R in synovial cells (SC) of rheumatoid arthritis (RA) patients has not previously been documented. This study conclusively evidences H4R expression in SC of RA patients by use of RT-PCR method. As RA consists mainly of immunological disturbances in SC of RA patients, this study’s findings document a novel histamine action site, and opens potential new avenues to investigate mechanisms and to develop pharmacotherapeutic agents for the disease.

Key words histamine H4 receptor (H4R) expression; synovial cell; rheumatoid arthritis

Histamine is a biological amine that affects a variety of functions in the human body. It has been known to participate in the mechanisms of inflammatory reaction, gastric acid secretion, and neurotransmission. To date, four subtypes of histamine receptor, i.e. H1 receptor (H1R), H2 receptor (H2R), H3 receptor (H3R), H4 receptor (H4R), have been identified. Relatively a new comer, the H4R, was identified in the year 2000. A plethora of expressions of H4R in organs in the immune system—such as the spleen, thymus, and leukocytes—implicated H4R in immune system control mechanisms. Histamine has been implicated in rheumatoid arthritis (RA). RA consists mainly of synovial tissue inflammation that may culminate throughout the body, but its molecular etiology remains unclear. Infiltration of macrophage and excessive formation of fibroblasts cause secretion of a variety of cytokines from synovial membranes in RA patients, and this in turn stimulates osteolytic activities.3) Histamine H4 receptor (H4R) expression; synovial cell; rheumatoid arthritis

The presence of H1R and H2R in human synovial cell culture (HSCC) has been shown clearly by ligand binding experiments. However, there has been no definitive evidence or conclusive report of the presence of H3R and H4R in HSCC. Therefore, by utilizing our expertise in RT-PCR technique, we examined the H4R specific mRNA expression in the HSCC obtained from 11 RA patients who underwent artificial knee replacement surgery at the Chiba University Hospital. This research work was performed in compliance with the procedures established by the University Bioethical Committee.

After excising the synovial membrane specimen under aseptic conditions, the sample was treated with 0.1% collagenase and 0.25% trypsin solutions to separate it into single cells. The cells were cultured in DMEM containing 12% FBS under 5% CO2 at 37 °C for 2 weeks. When the culture reached confluency, cells were harvested and the total RNA was extracted by using RNeasy Mini Kit with RNase-Free DNase Set (Qiagen, Hilden, Germany). The expression of HR specific mRNA was analyzed by the RT-PCR method using the RNA PCR Kit (AMV) ver. 2.1 (TaKaRa, Shiga, Japan). Unless specified otherwise, the basic protocol contained in the kits was followed by using the reagents in the kits. The sense and antisense primers used were: H1R (5'-TAAGCTGAGGCAGAGAACC; 5'-TACTGCTTGAATGCAGGC), H2R (5'-GACAGGCTCCATCTTACAACC; 5'-CTCTATTGGAGCTCATCCT), H3R (5'-TCTCTGCTCTTAACATCG; 5'-ATCATGAGCCGTTGATG), H4R (5'-ATCCCCCTACGCTGTGC; 5'-GAGGAAATCTCTGTCGATG), all of which were from Invitrogen (Garland, CA, U.S.A.), and internal controls, GAPDH (5'-TGACCTTGCCACAGCTTGC; 5'-CATCCATCTTCTCAGGA), was from Sigma Genosys (St. Louis, MO, U.S.A.). The extracted total RNA sample, in the amount of 2 µg, was reverse transcribed in the final volume of 20 µl that contained all of the necessary reagents supplied in the kit. The reaction temperatures in the PCR Thermal Cycler (TaKaRa, Shiga, Japan) were at 42 °C for 30 min, at 99 °C for 5 min and then at 55 °C for 5 min. Aliquots of 5 µl cDNA obtained from the RT reaction were amplified in the final volume of 25 µl of RNA PCR buffer containing the reagents supplied in the kit and 0.2 µl of appropriate sense and antisense primers. The mixture was incubated at 95 °C for 5 min and then subjected to the specific annealing cycles for each HR described below. One cycle consisted of 30 s at 95 °C, 30 or 45 s at respective annealing temperature, and 30 s at 72 °C. Then finally they were incubated at 72 °C for 5 min. The specific annealing cycles were as follows: for H1R and H2R, 35 cycles at 55 °C for 30 s; for H3R, 35 cycles at 55 °C for 45 s; for H4R and GAPDH, 35 cycles at 60 °C for 30 s. The PCR product was electrophoresed on 3% agarose gel, using 100b DNA Ladder (TaKaRa, Shiga, Japan) for bp size marker, and stained with ethidium bromide. The separated band sizes and intensities were visualized by UV trans-illumination. For positive controls 2.5 ng each of HR subtype specific mRNA standards (generous gifts from Professor Y. Masuho of Tokyo University of Science, Chiba, Japan) were loaded on the agarose gel.
different RA patients (RA1 and RA2). The gel was loaded with 5 μl of the amplified product; 1: 1 μl of PCR product; 2: 2 μl of PCR product + 8 μl of distilled water (DW) and no PCR product. S: specific mRNA Standard (2.5 ng); C: control (10 μl of distilled water (DW) and no PCR product). 1: 1 μl of PCR product + 9 μl DW; 2: 2 μl of PCR product + 8 μl of DW, etc. (b) Expressions of 4 subtypes of HR (H1R, H2R, H3R and H4R) specific mRNAs from 2 different RA patients (RA1 and RA2). The gel was loaded with 5 μl of amplified products. S: standard; C: control.

The gel was loaded with 5 μl of amplified products. S: standard; C: control; RA: RA patient. RE (relative expression): The relative expression of mRNA was calculated by normalizing the separated sample intensity value to the corresponding internal control (GAPDH) intensity value as 100%. The intensity values were measured by the use of image analyzer (IX81, OLYMPUS, Nagano, Japan).

(a) Determination of the optimum amount of the amplified H4R specific mRNA to be loaded on agarose gel. As a preliminary experiment RT-PCR was carried out through the amplification step, then loaded the gel with different amounts of the product as follows: S: specific mRNA Standard (2.5 ng); C: control (10 μl of distilled water (DW) and no PCR product). 1: 1 μl of PCR product + 9 μl DW; 2: 2 μl of PCR product + 8 μl of DW, etc. (b) Expressions of 4 subtypes of HR (H1R, H2R, H3R and H4R) specific mRNAs from 2 different RA patients (RA1 and RA2). The gel was loaded with 5 μl of amplified products. S: standard; C: control.

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Fig. 1. Culture of Synovial Cells Obtained from RA Patient

The photomicrograph is a representative sample of a culture before reaching confluency in the culture dish. (bar=200 μm)

Fig. 2. Expressions of HR Subtype Specific mRNA in HSCC from RA Patients

plate. Other pertinent protocols are described in the legends under appropriate Figs.

The primary cell cultures were monitored for opportunistic contaminations by mast cells and eosinophils in particular throughout the cell growth period up to the time of harvest for mRNA extraction (Fig. 1). All of the cells attached to the dish consisted of angular or spindle shapes, which are characteristic of fibroblasts. The viewing fields were remarkably clean and totally free of spheroid shaped cells that may suggest the presence of mast cells and eosinophils. In a preliminary experiment HSCC from RA patient No. 1 (RA1) was processed for the analysis of H4R through the amplification step, and then different amounts of the amplified product were loaded on the agarose gel. This preliminary test established that the conditions of experiment were adequate, and less than 10 μl of the product would be sufficient for detection of H4R specific mRNA (Fig. 2a). Analysis of the 4 subtypes of HR specific mRNA expressions in HSCC from 2 individual RA patients (RA1 and RA2) showed that under the established conditions of experiment H1R-, H2R- and H4R-specific mRNAs were expressed, but the H3R-specific mRNA was absent (Fig. 2b). The presence of H1R and H2R in HSCC has previously been reported.5) However, the detection of H4R-specific mRNA expression in HSCC has not been reported previously. Analyses of HSCC from 11 different RA patients (RA1 through RA11) confirmed the expression of H4R-specific mRNA in all of the samples from the RA patients (Fig. 3). Notably, the intensities of the separated H4R specific mRNA bands on the gel varied considerably from one to another, suggesting that there were differences in the cellular concentrations of H4R among the RA patients.

H4R is expressed in many organs of the human body such as the brain, heart, liver, and lungs.7,8) However, noticeably abundant expressions of H4R, especially in the spleen, leukocytes and thymus—the organs of the immune system—have been reported.9) The chemotaxis of mast cells and eosinophils are stimulated by histamine through H4R.10,11) IL-5 induces H4R specific mRNA in HL-60, a human promyelocytic leukemia cell line.12) H4R controls the release of IL-16 from CD8 positive T-lymphocytes.13) These observations suggest that H4R participates in immune reactions that underlie mechanisms of inflammation and allergy. RA is a disease arising from disturbances in the immune system; as such, H4R is likely to have a significant role in the disease. The present study further reveals that the extent of H4R expression varies considerably from patient to patient.

This report is confined to the discovery of H4R specific mRNA expression in HSCC. However, it is tempting to speculate that a considerable variation in the degrees of H4R specific mRNA expression among the RA patients (Fig. 3) suggested that H4R level may be related to the severity and duration of RA in the patients. The identification of H4R expression in the synovial system evidences a previously undetected site and mode of histamine action. This seminal finding introduces pertinent, new avenues to explore and to study the molecular etiology and progression of RA.

REFERENCES

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