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Lack of Detection of Human Retrovirus-5 Proviral DNA in Synovial Tissue and Blood Specimens From Individuals With Rheumatoid Arthritis or Osteoarthritis

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Abstract

Objective—Prior studies have suggested an association of human retrovirus 5 with rheumatoid arthritis. The purpose of this study was to determine if human retrovirus-5 proviral DNA is present in synovial tissue and blood specimens from patients with rheumatoid arthritis or osteoarthritis, or those without joint disease.

Methods—Synovial tissue and whole blood from 75 patients with rheumatoid arthritis, 75 patients with osteoarthritis, and 50 patients without a primary arthritis diagnosis were assayed by real-time quantitative polymerase chain reaction (PCR) using primers that amplify a 186-bp fragment of human retrovirus-5 proviral DNA.

Results—A total of 200 tissue specimens, 200 mononuclear cells, and 196 of 200 granulocyte specimens tested negative for human retrovirus-5 proviral DNA. No association between human retrovirus 5 and rheumatoid arthritis or osteoarthritis ($P = 0.516$) was identified. Granulocyte specimens from 4 patients, 2 with rheumatoid arthritis and 2 with osteoarthritis, yielded a low positive human retrovirus-5 proviral DNA signal (83–1,365 copies of human retrovirus-5 proviral DNA/ml blood).

Conclusion—Contrary to prior reports, we did not find an association between human retrovirus 5 and rheumatoid arthritis or osteoarthritis using a real-time PCR assay. Our findings are consistent with the recent finding that human retrovirus 5 is actually rabbit endogenous retrovirus H.

Keywords

Human retrovirus-5; Rheumatoid arthritis; Osteoarthritis

INTRODUCTION

In 1997, a 932-bp novel retrovirus fragment was cloned by reverse transcriptase polymerase chain reaction (PCR) from the salivary gland tissue of a patient with Sjögren's syndrome (1).

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The sequence was ascribed to an infectious agent genome belonging to an agent named human retrovirus 5 (HRV-5).

As a result of the finding of this sequence in a patient with Sjögren's syndrome, a subsequent study explored the hypothesis of an association of HRV-5 with Sjögren's syndrome. However, HRV-5 proviral DNA was identified in only 2 of 92 salivary gland tissues (55 from patients with Sjögren's syndrome and 37 from patients with non-Sjögren's syndrome) (2). One of the positive salivary gland tissues was from a patient who had sicca symptoms but did not satisfy the criteria for diagnosis of Sjögren's syndrome (2). The other was from a patient with secondary Sjögren's syndrome and rheumatoid arthritis (RA) (2). Although an association of HRV-5 with Sjögren's syndrome was not identified, the finding of HRV-5 in a patient with RA raised the question of an association of HRV-5 with RA. Supporting this association, Griffiths and colleagues detected HRV-5 proviral DNA in 12 (48%) of 25 synovial samples from patients with RA (3). Notably, however, they also detected HRV-5 proviral DNA in 3 of 5 synovial samples from patients with osteoarthritis (OA) (3).

In the present study, we examined the hypothesis that there is an association of HRV-5 with RA, and we further explored a possible association of HRV-5 with OA.

PATIENTS AND METHODS

Patients

Synovial tissue and whole blood from 75 patients (10 men, 65 women) with RA, 75 patients (33 men, 42 women) with OA, and 50 patients (21 men, 29 women) without a primary arthritis diagnosis were collected in sterile containers. The median age was 61 years (range 18–82 years) for the patients with RA, 69 years (range 38–86 years) for patients with OA, and 64 years (range 33–87 years) for patients without a primary arthritis diagnosis. All patients with RA met the 1987 American College of Rheumatology (formerly the American Rheumatism Association) classification criteria (4) for this disease. OA was diagnosed by the participating rheumatologist/orthopedists based on clinical and radiographic findings. Controls were patients without a primary arthritis diagnosis who were undergoing surgery for trauma, cancer, or infection. Synovial tissue was collected at the time of joint surgery (performed for reasons other than this study). Whole blood was separated into mononuclear cells and granulocytes using Histopaque 1119 and 1077 (Sigma, St. Louis, MO). Specimens were stored at -70°C until subjected to DNA extraction and amplification. This study was approved by the Mayo Institutional Review Board (#1268-98).

DNA extraction

DNA was extracted using the QIAamp DNA mini kit (Qiagen, Valencia, CA). The HRV-5 *pol*-containing plasmid (pHRV5.1) (1) spiked into a negative synovial tissue or blood was used as a positive control. DNA extracted from a negative synovial tissue or blood specimen was included between every 2 specimens as a negative control. Suitability of DNA for PCR was verified using the LightCycler–Control Kit DNA (Roche Molecular Biochemicals, Indianapolis, IN), amplifying a 110-bp fragment of the human β -globin gene.

PCR for HRV-5

Specimens were assayed in duplicate by real-time quantitative PCR using the LightCycler Fast-start DNA Master SYBR Green I kit (Roche Molecular Biochemicals). Primers (forward primer: 5'-AGGACGGCGTATTATG; reverse primer: 5'-ACTGTGGTATCCACTTTG) that amplify a 186-bp fragment of HRV-5 proviral DNA, encoding a putative protease-like protein, were developed using LightCycler Probe Design Software, Version 1.0 (Roche Molecular Biochemicals). Each PCR consisted of 2 μl of extracted DNA in a total volume of 20 μl with

3 mM MgCl₂, 0.3 μM of each primer, and 0.05 units/μl thermolabile uracil *N*-glycosylase (Roche Diagnostics, Mannheim, Germany). Cycling parameters were 95°C for 10 minutes, followed by 50 cycles of 95°C for 15 seconds, 53°C for 5 seconds, and 72°C for 10 seconds. A melting curve was generated by measuring the fluorescent signal generated while raising the temperature slowly from 65°C to 95°C at 0.1°C/second. A total of 10,000 copies of pHRV5.1 were spiked into sterile water, tissue, mononuclear cells, and granulocytes, and were extracted and analyzed. A product with a melting temperature of 84.3°C was detected and sequenced. The sequence matched the published HRV-5 proviral DNA sequence (GenBank AF480924). Extracted sterile water, tissue, mononuclear cells, and granulocytes alone did not yield amplified product. Serial 1:10 dilutions of pHRV5.1 spiked into tissue, mononuclear cells, and granulocytes were used as standards to produce a standard curve for tissue, mononuclear cells, and granulocytes, respectively (data not shown). The LightCycler software was used to calculate a concentration for positive samples. The mean ± SD PCR efficiency (calculated from the slope of the standard curve) was 1.93 ± 0.05 for tissue, 1.92 ± 0.05 for mononuclear cells, and 1.95 ± 0.05 for granulocytic cells.

Nucleic acid sequencing

Positive specimens were subjected to bidirectional sequencing (5) using the PCR primers as sequencing primers. DNA sequencing was performed by ABI PRISM Big Dye Terminator cycle sequencing with the ABI PRISM 377 automated DNA sequencer (Applied Biosystems, Foster City, CA). Sequence data were analyzed using Sequencher 4.1.4 (GeneCodes, Ann Arbor, MI). Fisher's exact test (2-tailed) was used for statistical analysis.

RESULTS

Detection of HRV-5 DNA

All 600 patient specimens (200 mononuclear cell specimens, 200 granulocyte specimens, and 200 tissue specimens) were tested in duplicate. All were positive for human β-globin. All 200 tissue specimens and all 200 mononuclear cells tested negative for HRV-5 proviral DNA, and 196 of 200 granulocytic cell specimens tested negative for HRV-5 proviral DNA. There was no association between HRV-5 and RA or OA ($P = 0.516$).

Four granulocytic cell specimens, 2 from patients with OA and 2 from patients with RA, yielded a positive signal for HRV-5 proviral DNA. All 4 were detected at a low (83–1,365) number of copies of HRV-5 proviral DNA/ml blood. All 4 were subjected to bidirectional sequencing and showed 96–98% nucleotide identity to GenBank AF480926 (*Oryctolagus cuniculus* clone B rabbit endogenous retrovirus H, complete sequence).

DISCUSSION

We did not find an association between HRV-5 and RA or OA, as has been previously reported (3). Studies linking new infectious agents with human diseases must include appropriate controls, and specimens from controls must be collected and processed in the same way as specimens from diseased humans. Assays used to detect such agents must be accurate and controlled. Prior studies reporting detection of HRV-5 from human specimens used nested PCR (2,3), which could account for some of the positive results previously reported. Our PCR assay utilized a closed system not subject to the pitfalls of nested PCR.

Recently it has been shown that HRV-5 is actually rabbit endogenous retrovirus H (6). We hypothesize that experimental rabbit studies ongoing in our laboratory while the granulocyte specimens were being prepared account for the low level of HRV-5 proviral DNA detected in 0.7% of our specimens. Whereas tissue samples were immediately placed in the freezer without

processing, blood samples were processed prior to being frozen and could have been contaminated during this initial processing. Our lack of detection of an association of HRV-5 with RA is consistent with findings of Gaudin et al (7).

In conclusion, contrary to some prior reports, we did not find an association between HRV-5 and RA or OA using a real-time PCR assay.

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