Pityriasis Rosea Is Not Associated With Human Herpesvirus 7

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Objective: To examine the proposed association between pityriasis rosea and human herpesvirus 7 (HHV-7).

Design: A retrospective cross-sectional survey.

Setting: University medical center in Switzerland.

Patients: Thirteen patients with pityriasis rosea and 14 persons with normal skin (control subjects).

Main Outcome Measures: Detection of HHV-7–specific DNA sequences and antigen (85-kd phosphoprotein [pp85]) by nested polymerase chain reaction and immunohistochemical analysis, respectively.

Results: Human herpesvirus 7 DNA sequences and expression of the HHV-7–specific immunodominant pp85 antigen were found in 1 (8%) of 13 lesional skin biopsy specimens of pityriasis rosea. The prevalence of HHV-7 DNA sequences and antigens is even slightly lower in lesional skin of patients with pityriasis rosea than in clinically and morphologically normal skin of 14 control persons, in 2 of whom (14%) HHV-7 DNA sequences and antigens could be detected.

Conclusion: The low detection rate of HHV-7 DNA sequences and antigens argues strongly against a causative role for HHV-7 in the pathogenesis of pityriasis rosea.

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PITRIASIS ROSEA (PR) is an acute exanthematous inflammatory skin eruption occurring usually once per lifetime in the second or third decade. Many epidemiological and clinical features suggest a viral pathogenesis for PR. They include the preferential occurrence of PR in the spring and fall and sometimes in communities; occasionally fever as a prodromal symptom; the occurrence of a preceding herald patch followed by the appearance of multiple skin lesions with a characteristic distribution; the spontaneous regression of skin changes within 6 to 12 weeks without therapeutic intervention; and recurrences during immunosuppression, as is often observed in patients with viral diseases. Several viruses such as picornaviruses, parvoviruses, and herpesviruses were proposed as causative agents of PR, but despite the detection of viruslike particles in the herald patch of PR, none has been conclusively associated with PR. In 1997, Drago and colleagues reported on the detection of DNA sequences from human herpesvirus 7 (HHV-7) in skin biopsy specimens, peripheral blood mononuclear cells (PBMCs), and plasma specimens of 12 patients with PR by using a nested polymerase chain reaction (PCR) protocol. In addition, virus-like particles were detected in cell cultures inoculated with the cell-free supernatant from cultured PBMCs of patients with PR and identified as herpesviruses by electron microscopy. Based on their findings, the authors proposed that PR is a clinical presentation of HHV-7 reactivation.

Human herpesvirus 7 is a ubiquitous virus. Primary infection occurs early in childhood at a high incidence and, in some patients, is associated with episodes of exanthem subitum or febrile illness. After primary infection, the virus then persists in the organism, replicates in salivary glands, and is shed in the saliva. The virus is reactivated in recipients of kidney and bone marrow transplants and, together with human cytomegalovirus and human herpesvirus 6, may complicate the engraftment of transplanted organs. Other associations of HHV-7 infection or reactivation with diseases in adults have not been established until now. The purpose of this study was to confirm and extend the proposed association between HHV-7 infection or reactivation and PR. We examined lesional skin specimens from 13 patients with PR and included in our study a control group from whom clinically and histologically normal skin specimens were obtained. The presence of HHV-7 was detected simultaneously with 2 different and unrelated approaches—a nested PCR protocol and immunohistochemical analysis. For the latter, we took advantage of a monoclonal antibody to HHV-7 extensively characterized in previous studies from our laboratories. This antibody, Mab5E1, is directed to an immunodominant

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PARTICIPANTS AND METHODS

We examined 13 skin biopsy specimens of PR lesions (including 4 herald patches) from 13 patients with clinically and histologically proven PR and specimens of histologically normal skin from 14 subjects undergoing plastic surgery. In all cases, the diagnosis of PR was based on a consideration of clinical and histological features. Of 13 patients with PR, 12 showed the typical clinical manifestation, with the development of multiple patchy erythematous and scaling skin lesions within days or as much as 2 weeks after the occurrence of a primary plaque. The spontaneous resolution of the lesions within 2 months was observed in all patients. Only 1 patient presented with an atypical manifestation of multiple papular lesions, but the lesions showed histologically typical features of PR and regressed spontaneously after 6 weeks. The histological features of all specimens were compatible with the diagnosis of PR. The median age of patients with PR was 25.8 years (age range, 17-49 years), whereas the median age of the control subjects was 24.3 years (age range, 16-44 years).

On histological examination, all PR lesions showed spongiosis, focal parakeratosis, and some dyskeratotic cells of the epidermis and a mixed perivascular inflammatory infiltrate in the upper dermis. The median time between the onset of the disease and a skin biopsy was 9.3 days (range, 2-17 days). The tissue specimens were routinely fixed in 10% buffered formalin and embedded in paraffin. Informed consent was obtained from all patients. Blood specimens were not available for the evaluation of viral presence in serum or PBMCs.

POLYMERASE CHAIN REACTION

DNA was extracted by proteinase K digestion according to standard procedures as previously described. To avoid contamination and product carryover, the microtome blade was cleaned with xylene after each cut, and DNA extraction, PCR, and gel electrophoresis were done in separate laboratories. Successful amplification of a β-globin fragment (268 base pairs) indicated that the specimens were adequate for PCR analysis and that no inhibitors were present. The HHV-7–specific sequences were amplified by nested PCR with 2 sets of primers consisting of the HV7 and HV8 external primers and of HV10 and HV11 internal primers, as previously described. These primers were shown to amplify specifically HHV-7 DNA and not the DNA from other human herpesviruses. Positive controls consisted of DNA extracted from cord blood mononuclear cells infected with HHV-7 and skin biopsy specimens known to harbor HHV-7. The PCRs containing all PCR reagents and no DNA template and skin tissue proved previously not to contain HHV-7 DNA were included as “negative controls.” The amplification products were subjected to electrophoresis in a 2% agarose gel and stained with ethidium bromide.

The sensitivity of the nested PCR system was determined to be about 1 × 10^3-ng DNA by serial 10-fold dilutions of the amplimer of the first PCR, followed by the second nested PCR and analysis by agarose gel electrophoresis. The specificity of the amplified products was confirmed by direct sequence analysis (data not shown).

IMMUNOHISTOCHEMICAL TECHNIQUE

For immunohistochemical study, we used the monoclonal antibody Mab5E1, which is directed against a phosphorylated protein (85-kd phosphoprotein [pp85]) of the virion tegument of HHV-7. The tissue sections were deparaffinized with xylene and incubated with Mab5E1 (diluted 1:50) for 90 minutes at room temperature. After the sections were washed several times with phosphate-buffered saline solution, the antibody was detected using alkaline phosphatase–anti–alkaline phosphatase complex (Dako Corporation, Glostrup, Denmark) according to the manufacturer's protocol. The staining reaction was developed using new fuchsin as chromogen, resulting in a red staining. For counterstaining, hematoxylin solution was briefly added. As a negative control, primary antibody was replaced by phosphate-buffered saline solution.

EXPRESSION OF HHV-7–SPECIFIC ANTIGENS

The expression of HHV-7–specific antigen was detected only in the PR skin biopsy specimens that contained viral DNA sequences and in 1 of 2 specimens of normal skin harboring the HHV-7 DNA sequence. In all cases, the cells expressing HHV-7 viral antigen pp85 were perivascular cells. Based on morphologic features, the cells expressing HHV-7 antigen represent mononuclear inflammatory cells such as lymphocytes and histiocytes.

COMMENT

Human herpesvirus 7 DNA sequences and cells infected with HHV-7 are present in lesional skin specimens of patients with PR and in clinically and histologically normal skin specimens of healthy persons at comparable levels (1 of 13 persons and 2 of 14 persons, respectively) and with an overall low incidence. The reliability of our experimental approach stems from the findings that PCR and immunohistochemical analysis concordantly detected HHV-7 in the same specimens—those with disease and specimens of normal skin. Current
results sharply contrast with those of Drago et al., who found HHV-7 DNA sequences in all skin and PBMC specimens analyzed and in plasma specimens from patients with PR at a higher frequency than from healthy persons.

The reasons for the discrepancy are unclear. Whereas in our study, authenticity of the PCR data was provided by sequencing of the amplified fragments and was substantiated by immunohistochemical analysis, PCR was the only analytic technique used in the preceding study. So whether DNA sequences other than those of HHV-7 were amplified cannot be excluded, in particular because skin specimens of healthy persons were not investigated as negative controls and, furthermore, because PCR amplification products were not sequenced. The PCR is well known to produce false-positive results due to contamination. Recently, in a PCR-based study on the presence of human herpesvirus 8, contamination occurred even in circumstances in which the control experiments did not indicate contamination of investigated specimens. In the preceding study, the presence of HHV-7 in cell-free plasma specimens from patients with PR was interpreted to support a causal relationship. In our study, plasma and PBMC specimens were not available for the detection of viral sequences. For a pathogenic association between HHV-7 and PR, however, the virus is expected to be present in the lesions. Given that in our study, HHV-7 DNA and antigens were not detected in PR specimens at a frequency compatible with a pathogenic association, the presence of viral DNA in plasma or in PBMCs would have been indicative only of viral replication at sites other than those involved by the disease under examination rather than of a causal relationship. Alternative explanations for the presence of viral DNA in serum are conceivable. Thus, HHV-7 reactivation may be the consequence of a transient immunodepressive state, as is often observed with herpesviruses. For example, herpes labialis and herpes zoster lesions are frequently observed with herpesviruses. For example, herpes labialis and herpes zoster lesions are frequently observed. 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**CONCLUSIONS**

Inasmuch as a consistency of findings from different groups of investigators is crucial to demonstrate a causal association between an infectious agent and a disease, current results argue strongly against a causative role for HHV-7 in the pathogenesis of PR.