

Pityriasis Rosea is Associated with Systemic Active Infection with Both Human Herpesvirus-7 and Human Herpesvirus-6

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Pityriasis rosea is a common skin disease that has been suspected to have a viral etiology. We performed nested polymerase chain reaction to detect human herpesvirus-7, human herpesvirus-6, and cytomegalovirus DNA in lesional skin, nonlesional skin, peripheral blood mononuclear cells, serum, and saliva samples isolated from 14 pityriasis rosea patients. Viral mRNA expression and virion visualization within lesional skin were studied by *in situ* hybridization and transmission electron microscopy, respectively. By nested polymerase chain reaction, human herpesvirus-7 DNA was present in lesional skin (93%), nonlesional skin (86%), saliva (100%), peripheral blood mononuclear cells (83%), and serum (100%) samples, whereas human herpesvirus-6 DNA was detected in lesional skin (86%), nonlesional skin (79%), saliva (80%), peripheral blood mononuclear cells (83%), and serum (88%) samples. By contrast, cytomegalovirus DNA was not detected in these tissues. Control samples from 12 healthy volunteers and 10 psoriasis patients demonstrated rare

positivity for either human herpesvirus-7 or human herpesvirus-6 DNA in skin or serum. By *in situ* hybridization, infiltrating mononuclear cells expressing human herpesvirus-7 and human herpesvirus-6 mRNA were identified in perivascular and periappendageal areas in 100% and 75% pityriasis rosea skin lesions, respectively, compared to herpesviral mRNA positivity in only 13% normal skin and psoriasis skin controls. Transmission electron microscopy failed to reveal herpesviral virions in pityriasis rosea lesional skin. Nested polymerase chain reaction and *in situ* hybridization enabled detection of human herpesvirus-7 and human herpesvirus-6 in skin and other tissues isolated from patients with pityriasis rosea. These results suggest that pityriasis rosea is associated with systemic active infection with both human herpesvirus-7 and human herpesvirus-6. **Key words:** herpesviruses/*in situ* hybridization/ pathogenesis/ polymerase chain reactivation *J Invest Dermatol* 119:793–797, 2002

Pityriasis rosea (PR) is a common inflammatory skin disease that usually occurs in the second or third decade of life. The initial skin lesion is called a “herald patch”, and usually appears on the trunk as a 2–3 cm oval scaly plaque with a central salmon-colored area and a darker erythematous peripheral zone (**Fig 1**). The herald patch is typically followed 1–2 wk later by the appearance of numerous smaller, oval, erythematous, scaly, slightly pruritic plaques that tend to occur in a “Christmas tree” pattern on the trunk (**Fig 1**). The disease normally resolves spontaneously within 4–8 wk. Although the etiology is unknown, several clinical and epidemiologic features suggest that an infectious agent is involved in PR pathogenesis, including seasonal variation, occasional constitutional symptoms (fever, malaise, loss of appetite), and reported clustering in families or communities (Parsons, 1986; Allen *et al*, 1995).

In 1997, Drago *et al* first suggested that reactivation of human herpesvirus-7 (HHV-7), a ubiquitous β -herpesvirus, was linked to PR (Drago *et al*, 1997a). Others have not been able to replicate these results, however (Kempf *et al*, 1999; Watanabe *et al*, 1999; Yasukawa *et al*, 1999; Kosuge *et al*, 2000; Offidani *et al*, 2000; Chuh and Peiris, 2001; Chuh *et al*, 2001; Wong *et al*, 2001). Reactivation of HHV-6, another common β -herpesvirus closely related to HHV-7, was also recently claimed to be associated with PR (Yasukawa *et al*, 1999). To address this issue, we examined lesional skin, nonlesional skin, saliva, peripheral blood mononuclear cells (PBMC), and serum samples from 14 patients with PR for the presence of HHV-7 and HHV-6 DNA and mRNA.

MATERIALS AND METHODS

Patients and sample collection Fourteen patients with PR were referred to the outpatient clinic at the NIH Clinical Center between February 1997 and December 2000. The National Cancer Institute Institutional Review Board approved the protocol and informed consent was obtained from all PR patients, healthy volunteers, and psoriasis patients. The diagnosis of PR was based on the prototypic clinical manifestations (as described above) and confirmed by classic histologic findings, including irregular acanthosis, focal parakeratosis, and superficial perivascular infiltration of lymphocytes and histiocytes (**Fig 2A–F**).

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Abbreviations: HHV, human herpesvirus; PBMC, peripheral blood mononuclear cells; PR, pityriasis rosea.

Punch biopsy specimens were obtained from lesional and nonlesional skin during the acute phase of the illness. In most, but not all, PR patients, blood and saliva samples were also collected. In addition, normal skin, blood, and saliva samples were obtained from 12 healthy age- and sex-matched volunteers and lesional skin was biopsied in 10 adult psoriasis patients with a mean age of 44.9 y and an age range of 28–55 y. Psoriasis, a common prototypic inflammatory skin disease, was chosen as a control disease for PR because psoriatic lesions contain numerous CD4⁺ T cells, which are cells known to harbor HHV-7 and HHV-6. PBMC were isolated by density gradient centrifugation of heparinized whole blood. After centrifugation of clotted blood, sera were passed through 0.45 μ m filters.

Nested polymerase chain reaction (PCR) To maximize PCR sensitivity, DNA was extracted from freshly obtained tissue samples using QIAamp DNA Mini Kits (Qiagen, Valencia, CA) immediately after sample collection. To avoid contamination, DNA extraction, PCR, and gel electrophoresis were done in separate laboratory locations using separate sets of equipment. The following primer sets were used for nested PCR to amplify human herpesviral DNA sequences (5'–3'): CMV-1 (outer sense), TTT CCA AGT CTC CAC CCC AT; CMV-2 (outer antisense), GTA CTT ACG TCA CTC TTG GC; CMV-3 (inner sense), GGG AGT TTG TTT TGG CAC CA; CMV-4 (inner antisense), CGC GTT CCA ATG CAC CGT TC; HHV-6-1 (outer sense), AAG CTT GCA CAA TGC CAA AAA ACA G; HHV-6-2 (outer antisense), CTC GAG TAT GCC GAG ACC CCT AAT C; HHV-6-3 (inner sense), TCC ATT ATT TTG GCC GCA TTC GT; and HHV-6-4 (inner antisense), TGT TAG GAT ATA CCG ATG TGC GT. For HHV-7, two sets of primers were used. One primer set has been described previously (Drago *et al*, 1997a), although some modifications were made to allow for amplification for both JI and RK strains of HHV-7. Specifically, the outer antisense primer was changed from CAC AAA AGC TCG CTA TCA A to CAC AAA AGC [A/G]TC GCT ATC AA. The other HHV-7 primer set was HHV-7-1 (outer sense), TTT TTA CAT TTG GCT TGC TTT TTG; HHV-7-2 (outer antisense), ATA TTT CTG TAC CTA TCT TCC CAA; HHV-7-3 (inner sense), TGC TTT TTG GTT TGT AAA TTC; and HHV-7-4 (inner antisense), GAA TTT ATG GAG TTT GGT CTG. For positive viral DNA controls, CMV, HHV-6, and HHV-7 purified DNA were purchased (Advanced Biotechnologies, Columbia, MD). To evaluate for potential primer/reagent contamination, tubes that contained all reagents except target DNA were prepared each time that PCR was performed. A total of 70 cycles of nested PCR (35 \times 2) was used as described previously (Blauvelt *et al*, 1997). Amplified PCR products were electrophoresed within 1.5% agarose gels and visualized by ultraviolet light fluorescence after ethidium bromide staining. All PCR reactions were repeated twice.

In situ hybridization (ISH) Five micrometer thick formalin-fixed paraffin-embedded sections were placed on slides coated with 3-aminopropyl-triethoxysilane. Two sections from each tissue per patient were hybridized with a ³⁵S-labeled, single-stranded, antisense RNA probe of HHV-6 U48 and HHV-7 U10. Previous southern blot analyses and ISH experiments showed no cross-reactivity between the two probes that contain each of these genes (Berneman *et al*, 1992). The lengths of these

riboprobes were 969 bp and 874 bp, respectively. They were generated by PCR using the following primers: HHV-6 U48 (sense), GGA gga tcc



Figure 1. Typical clinical features of PR showing a herald patch on the arm and secondary oval plaques on the abdomen.

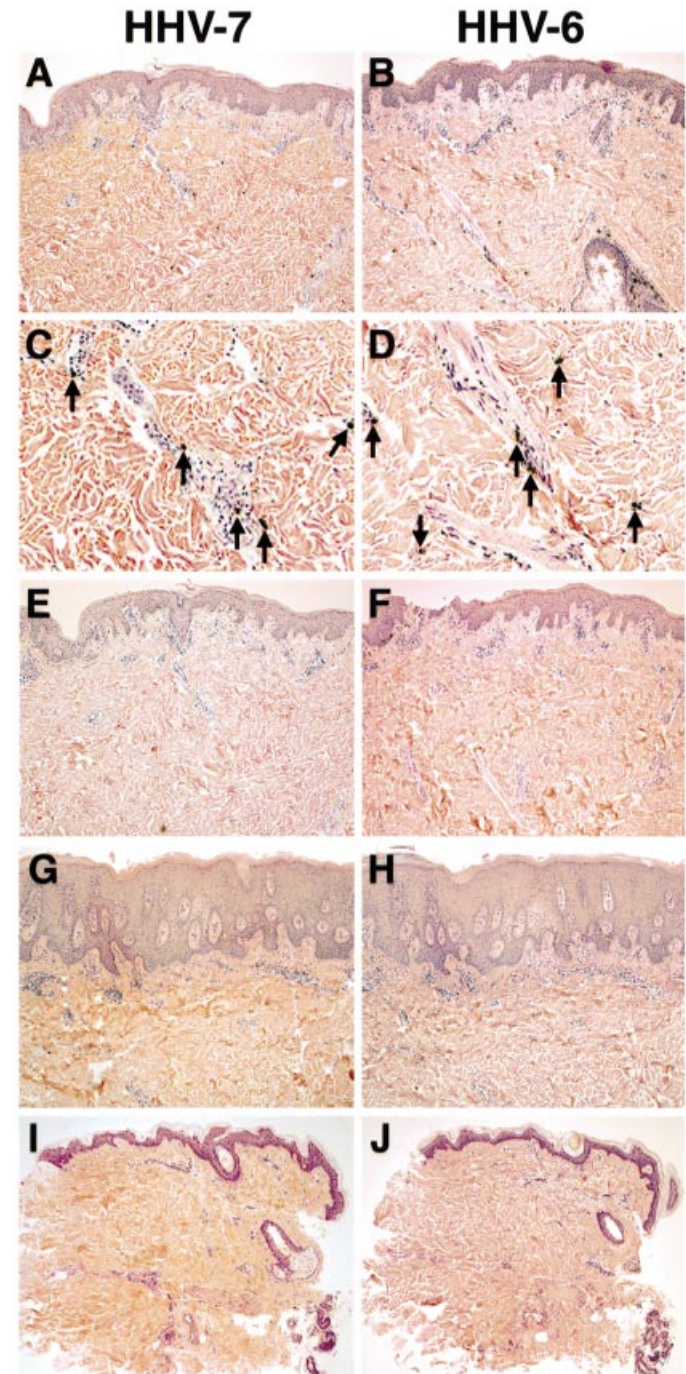


Figure 2. Detection and localization of HHV-7 and HHV-6 mRNA in skin lesions of PR by ISH. (A, C) HHV-7 mRNA expression (black silver grains, as indicated by arrows in C) in infiltrating mononuclear cells in perivascular and periappendageal areas (antisense probe). (E) Skin from the same site reveals no hybridization with a control HHV-7 sense probe. No infected cells are found in skin from a psoriasis patient (G, antisense probe) or a healthy volunteer (I, antisense probe). (B, D) HHV-6 mRNA expression (black silver grains, as indicated by arrows in D) in infiltrating mononuclear cells in perivascular and periappendageal areas (antisense probes). (F) Skin from the same site reveals no hybridization with a control HHV-6 sense probe. No HHV-6 mRNA is detected in skin from a psoriasis patient (H, antisense probe) or a healthy volunteer (J, antisense probe). Magnification: (A), (B), (E)–(J), 50 \times ; (C), (D) 100 \times .

GAA CAA CTA TGC TCC TCC GA; HHV-6 U48 (antisense), CCT gga tcc CTC TTA ACA CGG GCA TTG GT; HHV-7 U10 (sense), CGC gga tcc CGC CCT AAG TAA CTA CAC TT; HHV-7 U10 (antisense), TGG gga tcc AGT TTG GGA GAT TCT CTG GA (BamHI site is in lowercase letters). Cytospin preparations of either HHV-6 or HHV-7 infected SupT1 cells were used as positive controls and to rule out cross-reactive hybridization. Sense-strand probes were used as negative controls. De-waxed paraffin sections were boiled in citrate buffer pH 6.0 for 5 min, chilled down to room temperature, rinsed in water containing 2% diethyl pyrocarbonate, and prehybridized for 2 h at 45°C. The prehybridization mixture consisted of 50% formamide, 0.5 M NaCl, 10 mM Tris-HCl at pH 7.4, 1 mM ethylenediamine tetraacetic acid, 0.02% Ficoll-polyvinyl pyrrolidone, and 2 mg per ml tRNA. Prehybridization was followed by incubation with the hybridization mixture (prehybridization mixture, 10% dextran sulfate, and 2 × 10⁶ dpm per ml probe) overnight at 45°C in a moist chamber. After several washings in standard saline citrate (SSC), the sections were digested with ribonuclease at 37°C for 40 min, washed again in 2 × SSC, dehydrated, and dipped in Kodak NTB-2 emulsion. Exposure at 4°C was for 7 d. After development in Kodak D-19, sections were counterstained with hematoxylin, mounted, and examined with an Axiophot Zeiss microscope. All viral mRNA positive cells were counted in two similarly stained sections.

Transmission electron microscopy (TEM) Fresh lesional skin obtained by punch biopsy from nine PR patients was immediately placed in neutral buffered 2.5% glutaraldehyde at room temperature and allowed to fix at least overnight. Tissue was postfixed in 1% OsO₄, dehydrated in graded ethanol and propylene oxide, and embedded in Spurr's epoxy. Semi-thin plastic sections (1 μm) were cut with glass knives and stained with combined methylene blue, azure II, and basic fuchsin stain. Thin sections were stained with uranyl acetate and lead citrate and examined on a LEO EM10 transmission electron microscope operating at 60 kV.

Statistical analyses Comparison of the PCR positivity was by χ² tests with Yates's correction. Comparison of the number of cells expressing viral mRNA was made by Wilcoxon sum tests. A p-value of less than 0.05 was considered to be statistically significant.

RESULTS

There were eight men and six women whose mean age was 35.4 y (range, 16–58 y). All of the patients had clinical and histologic findings of “classic” PR (**Figs 1, 2A–F**); no atypical clinical presentations were noted. DNA was isolated from lesional and nonlesional punch biopsies of the skin from all 14 patients. DNA from other tissues (serum, PBMC, saliva) was collected and considered “evaluable” if human β-globin DNA could be amplified by nested PCR and visualized by gel electrophoresis.

HHV-7 DNA was present in 13 of 14 (93%) lesional skin, 12 of 14 (86%) nonlesional skin, 10 of 10 (100%) saliva, 10 of 12 (83%) PBMC, and 8 of 8 (100%) serum samples (**Table I**). The percentages for detecting HHV-6 DNA in these same tissues were similar, including 12 of 14 (86%) lesional skin, 11 of 14 (79%)

Table I. Nested PCR detection of β-herpesviral DNA in fresh tissue^a

	PR	Psoriasis	Healthy volunteer
HHV-7			
Lesional	93%	0%	N/A
Nonlesional	86%	N/A	25%
Saliva	100%	N/A	92%
PBMC	83%	N/A	82%
Serum	100%	N/A	0%
HHV-6			
Lesional	86%	10%	N/A
Nonlesional	79%	N/A	17%
Saliva	80%	N/A	42%
PBMC	83%	N/A	36%
Serum	88%	N/A	0%

^aCMV DNA was not detected in PR tissues, except for one PR lesional skin sample. N/A, not available.

nonlesional skin, 8 of 10 (80%) saliva, 10 of 12 (83%) PBMC, and 7 of 8 (88%) serum samples (**Table I**). CMV DNA was negative in all but one of the samples examined (**Table I**). The frequency of detecting either HHV-7 or HHV-6 in skin and serum samples from PR patients was significantly higher than for healthy individuals and patients with psoriasis (p < 0.01). As expected, detection of HHV-7 or HHV-6 DNA in saliva and PBMC samples (a sign of prior infection) was not significantly different in PR patients and healthy volunteers (p > 0.05). To rule out the possibility of cross-reactivity between HHV-7 and HHV-6 (a common problem), HHV-7 viral DNA PCR controls were always positive using HHV-7 primer sets and always negative using the HHV-6 primer set, and HHV-6 viral DNA PCR controls were always positive using the HHV-6 primer set and always negative using the HHV-7 primer sets (data not shown). Importantly, positive PCR bands were never observed in the no DNA control tubes.

Due to cost considerations, ISH analyses were performed on only a subset of randomly selected skin samples obtained from PR patients, healthy volunteers, and psoriasis patients. Using HHV-7 specific antisense probes, HHV-7 mRNA was detected in eight of eight PR lesional skin specimens by ISH, but in only one of eight skin biopsies from controls (p < 0.01, **Table II, Fig 2**). Positive cells appeared to be infiltrating mononuclear cells located mainly around blood vessels and appendages (**Fig 2A, C**). ISH using HHV-7 sense probes were always negative in PR specimens (**Fig 2E**). Using the same eight specimens with HHV-6 specific antisense probes, the expression of HHV-6 mRNA was also observed in six of eight PR skin specimens by ISH, but in only one of eight skin biopsies from controls (p < 0.01, **Table II, Fig 2**). Similar to HHV-7 ISH, the morphologic features of cells expressing HHV-6 mRNA appeared to be mainly mononuclear cells scattered in perivascular and periappendageal regions (**Fig 2B, D**). No HHV-7 or HHV-6 positive cells were detected in epidermis. Overall, the number of HHV-6 mRNA positive cells was less than the number of HHV-7 mRNA positive cells detected in the same tissue (**Table II**). Nonlesional skin from PR patients was not available for ISH analyses because these tissue samples were used solely for DNA extraction/PCR studies. Importantly, ISH using HHV-7 specific antisense probes were always positive on HHV-7 infected SupT1 cells and negative on HHV-6 infected SupT1 cells; conversely, ISH using HHV-6 specific antisense

Table II. Number of HHV-7 and HHV-6 mRNA positive cells by ISH

	HHV-7		HHV-6	
	Perivascular	Periappendageal	Perivascular	Periappendageal
PR				
#1	10	14	4	10
#2	6	7	2	8
#3	11	5	0	0
#4	2	2	0	0
#5	8	10	3	5
#6	5	10	4	5
#7	6	10	1	2
#8	9	17	9	22
Psoriasis				
#1	0	0	0	0
#2	0	0	0	0
#3	0	0	0	0
#4	0	0	0	0
Healthy volunteer				
#1	0	0	0	0
#2	0	0	0	0
#3	0	0	0	0
#4	0	1	0	2

probes were always positive on HHV-6 infected SupT1 cells and negative on HHV-7 infected SupT1 cells (data not shown).

By TEM, the dermal appendages, nerves, and blood vessels were often rich in mononuclear cell infiltrate, lymphocytes, and macrophages. Exhaustive TEM examination of the dermis, including the infiltrates, of nine lesional PR specimens failed to reveal any viral particles, including herpesvirus group virions (data not shown).

DISCUSSION

In this study, we found clear evidence of systemic active infection with both HHV-7 and HHV-6 (two closely related β -herpesviruses) in patients with PR. Specifically, HHV-7 and HHV-6 DNA were readily and consistently found in cell-free serum samples obtained from PR patients. This finding is a sign of active viral replication. In addition, both HHV-7 and HHV-6 mRNA expression were documented in lesional skin of PR patients. Importantly, our report is the first to show evidence of viral gene expression and localization within PR skin lesions. The fact that we detected HHV-7 and HHV-6 DNA in saliva suggests that the patients were not experiencing primary infection with HHV-7 and/or HHV-6, as salivary gland cells are a reservoir for these herpesviruses only in previously infected individuals (Black and Pellett, 1999; Clark, 2000). As controls, we did not detect CMV DNA, another β -herpesvirus, in sera or skin from PR patients. Additionally, herpesviral DNA was not detected in sera from healthy volunteers and no HHV-7 or HHV-6 mRNA expression was detected in skin from healthy volunteers or from patients with psoriasis. Taken together, these controls indicate that CMV was not reactivated in PR patients and that HHV-7 and HHV-6 were not reactivated in healthy volunteers and patients with psoriasis.

Although Drago *et al* recently reported an association between HHV-7 and PR, these investigators did not document an additional role for HHV-6 in this disease, nor did they localize virus within PR skin lesions (Drago *et al*, 1997a, 1997b). Furthermore, reported studies on PR and HHV-7 following the initial report by Drago *et al* did not find evidence for an association between PR and HHV-7 (Kempf *et al*, 1999; Watanabe *et al*, 1999; Yasukawa *et al*, 1999; Kosuge *et al*, 2000; Offidani *et al*, 2000; Chuh and Peiris, 2001; Chuh *et al*, 2001; Wong *et al*, 2001). Thus, the body of published information on this topic has created a confusing picture. It should be pointed out that most of these negative studies that failed to find HHV-7 DNA in lesional PR tissue, plasma, or serum did not use nested PCR and/or extracted DNA from formalin-fixed paraffin-embedded tissue, methods that may have led to the negative results (Kempf *et al*, 1999; Yasukawa *et al*, 1999; Kosuge *et al*, 2000; Chuh and Peiris, 2001; Chuh *et al*, 2001; Wong *et al*, 2001). Regarding HHV-6, a recent study suggested a causal link to PR; however, these researchers did not implicate an additional role for HHV-7 in PR pathogenesis (Yasukawa *et al*, 1999).

Because of the disparate findings in the literature and the high degree of genetic similarity between HHV-7 and HHV-6, we were extremely cautious in our selection of virus-specific PCR primer sets, ISH antisense probes, and positive and negative control samples. We also chose to extract DNA only from fresh PR tissues (skin and others), use nested PCR to enhance detection sensitivity and specificity, and survey for all three types of known human β -herpesviruses (HHV-7, HHV-6, and CMV). Importantly, we also utilized mRNA ISH to confirm and extend our PCR results. Our results were striking and clear, providing firm evidence that both HHV-7 and HHV-6 are reactivated in patients with PR. Interestingly, HHV-7 reactivation has been reported to trigger reactivation of HHV-6 (Frenkel and Wyatt, 1992; Katsafanas *et al*, 1996; Tanaka-Taya *et al*, 2000), although the reverse has not been described. Because of this, we hypothesize that HHV-7 reactivation is able to subsequently reactivate HHV-6 in patients with PR. Our finding of a slightly lower frequency of detection of HHV-6 in PR tissue, compared to HHV-7, supports this hypothesis (Tables I,

II). As of now, however, it remains unclear as to what triggers active HHV-7 infection. This question will be a focus of further studies on PR.

Both HHV-7 and HHV-6 are known to infect and reside in circulating CD4⁺ lymphocytes (Lusso *et al*, 1988; Takahashi *et al*, 1989; Lusso *et al*, 1994). These cells are the likely source of cell-free viral DNA found in sera of PR patients. CD4⁺ lymphocytes, after migrating to skin, are also the likely source of viral mRNA that we observed by ISH (Fig 2). By contrast, we found no evidence of viral infection of resident skin cells by ISH or TEM, including keratinocytes, Langerhans cells, or fibroblasts (Fig 2 and data not shown). We believe our ability to detect virus by nested PCR and ISH (but not by TEM) reflects the low levels of HHV-7 and HHV-6 in PR lesional skin and the relatively low sensitivity of TEM to detect rare virion-producing cells. Indeed, our findings suggest that the skin lesions of PR are not due to direct infection of skin cells, but rather occur as a reactive response to systemic viral replication. The detection of elevated serum interferon- α levels in PR patients is consistent with this hypothesis (Drago *et al*, 1997b). It is also interesting to note that roseola, the disease of primary infection with HHV-6 (and sometimes HHV-7) in young children, is associated with a reactive viral exanthem, and not with a rash due to direct infection of skin cells (Asano *et al*, 1991).

Current standard of care for PR patients is to provide symptomatic relief of occasional pruritus using oral antihistamines and/or topical corticosteroids and to provide reassurance to patients that the PR rash will resolve spontaneously in 4–8 wk. Our study provides a rationale for additional therapeutic intervention. Specifically, it is possible that initiation of antiherpesviral therapy that targets HHV-7 and HHV-6 may hasten recovery from PR. Unfortunately, these particular herpesviruses are not sensitive to acyclovir and its derivatives (Takahashi *et al*, 1997; Yoshida *et al*, 1998; Black and Pellett, 1999). By contrast, ganciclovir, which does have significant antiviral activity against HHV-7 and HHV-6, is expensive and its use is associated with significant side-effects. Thus, therapeutic intervention with existing antiherpesviral drugs in PR patients does not seem warranted at this time.

In summary, we found that PR, a common skin disease suspected to be of viral etiology, is associated with systemic active infection with both HHV-7 and HHV-6, two common closely related human herpesviruses. We believe this is the most compelling report to date implicating PR as a viral exanthem. Our findings should lead to future studies on possible trigger(s) for herpesvirus reactivation in PR patients, as well as to antiherpesviral therapeutic studies designed to hasten recovery from PR.

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