INVESTIGATIVE REPORT

Detection of Human Herpesvirus 8 in the Skin of Patients with Pityriasis Rosea

Anestis PRANTSIDIS[1], Dimitrios RIGOPOULOS[2], Georgios PAPATHEODOROU[3], Panayiotis MENOUNOS[4], Stamatis GREGORIOU[2], Ifigeneia ALEXIOU-MOUSATOU[5] and Andreas KATSAMBAS[2]
Departments of Dermatology, [1]414 Military Hospital, Penteli, [2]University of Athens, "A. Sygros" Hospital, Athens, [3]Clinical Research Unit, 401 General Army Hospital, Athens, [4]Laboratory of Research, Nursing Military Academy, Athens and [5]Department of Microbiology, University of Athens, "A. Sygros" Hospital, Athens, Greece

Pityriasis rosea is an acute self-limiting dermatosis with clinical and epidemiological features that suggest viral involvement. The aim of this study was to investigate a possible association between pityriasis rosea and human herpesvirus 8 (HHV-8). Lesional skin tissue was obtained from 34 Kaposi's sarcoma-negative, immunocompetent patients with typical acute phase pityriasis rosea. Nested polymerase chain reaction with specific primer for HHV-8 DNA sequences was performed and all positive results were confirmed by sequencing. Seven out of 34 lesional skin specimens (20.5%) were found to be positive for the HHV-8 genome. All the positive samples were confirmed by DNA sequencing. We conclude that, in some cases, HHV-8 is implicated the pathogenesis of pityriasis rosea.
Key words: pityriasis rosea; human herpesvirus 8; pathogenesis; exanthema.

Pityriasis rosea (PR) is an acute, self-limiting papulo-squamous disease, which is common in adolescents and young adults, and usually lasts for 4–8 weeks. It has long been assumed to represent a delayed reaction to a viral infection, as indicated by seasonal clustering, occasional occurrence among family members, and a low probability of recurrence (1).

The aetiological factors involved in PR still remain vague and most authors consider a multifactorial disease with herpesviruses 6 and 7 playing an important role (2).

The aim of this study was to investigate the possible association between PR and human herpesvirus 8 (HHV-8).

PATIENTS AND METHODS

Thirty-four patients with PR (19 women and 15 men, age 15–58 years, median 33 years) participated in the study from 25 January 2005 to 10 March 2006 at the Department of Dermatology of "A. Sygros" Hospital, University of Athens, Greece. All patients had the typical clinical findings of PR, and were healthy and

immunocompetent. All cases were carefully selected to have the typical appearance (i.e. existence of herald patch, discrete circular or oval lesions scaling with central clearing, "Christmas tree" pattern of distribution) and all atypical forms were excluded. The presenting symptoms were fatigue, itch and skin irritability, and 12 patients described systemic symptoms, such as myalgias (8 patients), headache (6 patients), arthralgias (4 patients) and irritability (2 patients). Skin biopsy specimens were taken at the time of presentation, during the early acute phase (20 specimens) or the manifest phase of the disease (14 specimens). Skin biopsies were superficial, composed mainly of epidermis, all were lesional, mainly from the periphery of the lesions and were taken from the most recent peripheral lesions. Blood samples were also taken and venereal disease research laboratory, HIV examinations were performed. Histology examination was performed on a second, full-thickness lesional skin tissue biopsy from a close site, in order to confirm the clinical diagnosis. All patients were re-evaluated during the convalescent phase, and the typical clinical course of PR was confirmed in all patients until complete resolution. Informed written consent (i.e. that the investigation was performed for scientific reasons and had no effect on their clinical course) was obtained for all patients.

Control group

A total of 17 biopsy specimens from clinically and histologically normal skin were obtained from healthy volunteers with the same age and sex ratio as the study subjects, and were included as controls in the study. Written informed consent was also obtained from all the control subjects.

Polymerase chain reaction analysis

DNA was isolated from fresh frozen skin biopsy specimens using a commercial kit (QIAmp DNA Mini Kit, Qiagen Ltd, Crawley, UK) according to the manufacturer's instructions.

The 333-bp ORF 26 coding region from nucleotide position 258 to 590 was amplified by nested polymerase chain reaction (PCR) with an outside primer pair 5'-ATC-TAT-CCA-AGT-GCA-CAC-TCG-3' and 5'-CTG-GGA-ACC-AAG-GCT-GAT-AGG-3' and an inside primer pair 5'-GAT-GGA-TCC-CTC-TGA-CAA-CCT-3' and 3'-GGA-TCC-GTG-TTG-TCT-ACG-3'.

PCRs were performed in a final volume of 50 µl using a PCR processor (MJ Mini Cycler, MJ Research, Waltham, USA). Each PCR mixture contained 50 mM KCl, 10 mM Tris HCl, 0.1% Triton, 3 mM $MgCl_2$, 200 µM of each deoxynucleoside triphosphate (dNTP), 2.5 U Taq DNA polymerase (Promega Co., Madison, USA) and 25 pmol of each primer.

The cycling conditions for the first step (outside primers) were as follows: a 2-min activation step for the taq DNA polymerase, followed by 35 cycles of 1-min denaturation at 94°C, 1-min annealing at 50°C and 2-min elongation at 72°C. The last cycle was followed by a final 4-min extension step at 72°C.

The cycling profile for the second step (inside primers) was the same, except for an annealing temperature of 58°C.

A water blank and a DNA sample extracted from Kaposi's sarcoma skin biopsy were processed with each batch of clinical samples as negative and positive controls, respectively.

The products of nested PCR were analysed by electrophoresis on 2% agarose gels with ethidium bromide staining.

Sequencing of PCR products

PCR products were excised from 3% agarose gels, and DNA was isolated using QIAquick Gel Extraction Kit (Qiagen Ltd, Crawley, UK) according to the manufacturer's instructions. The products were sequenced on a 310 ABI Prism sequencer (PE Applied Biosystems, Foster City, USA) using 5 pmol of either forward or back primers.

RESULTS

Seven out of 34 specimens (20.5%) were found to be positive for HHV-8 genome (4 women, 3 men). Considering the time, 5 of them were taken during the acute phase, and 2 during the manifest phase of the disease. All the positive samples were confirmed by sequencing. All control samples were negative.

DISCUSSION

The role of HHV-6 and -7 in the pathogenesis of PR has been discussed for a long time. In 1997, Drago et al. (3, 4) reported the detection of DNA sequences from HHV-7 in skin biopsy specimens, peripheral blood mononuclear cells (PBMCs) and plasma specimens of all 12 patients with PR, using a nested 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. By contrast, in healthy individuals HHV-7 DNA was detected in no skin specimens, in only 44% of PBMC, and in none of the sera. These data indicated that HHV-7 reactivation was occurring during PR, and PR is the clinical presentation of this reactivation. A subsequent study failed to detect any HHV-7 DNA in 14 formalin-fixed paraffin-embedded tissues from lesional skin of PR patients (5). Many studies have followed these initial results, investigating the role of HHV-7 and HHV-6 separately and together in PR pathogenesis, with positive (6–10) or negative results (11, 12). The possibility of an individual, or a simultaneous action, or an interaction of the herpesviruses has also been studied. HHV-6 can be reactivated by an HHV-7 infection or reactivation, as some HHV-7 genes may transactivate those of HHV-6 and may stimulate HHV-6 replication and reactivation (6, 7, 9, 13). A recent study by Drago et al. (14) suggested that systemic acyclovir may hasten the recovery of patients with PR, providing further evidence for the viral aetiology of PR.

HHV-8 (or Kaposi's sarcoma (KS)) is considered to be a member of the *Gammaherpesvirinae* subfamily, presenting a high degree of homology with Epstein-Barr virus and *herpesvirus saimiri*. HHV-8 has become the most prominent candidate for the infectious aetiological cofactor of KS since its DNA sequence was discovered in tissue specimens and blood in all forms of KS. The infectious nature of HHV-8 has been confirmed by the isolation and propagation of a filterable agent from skin lesions of patients with AIDS-associated KS. This agent produced a cytopathic effect in cultured cells and revealed ultrastructural HHV-like features in their nuclei and cytoplasm. With regard to modes of transmission, studies suggest that, beside the sexual, it is possible that non-sexual routes of HHV-8 transmission (e.g. saliva) exist. In addition to KS, HHV-8 has been detected in patients with primary effusion lymphoma, a rare disease previously known as body cavity-based B-cell lymphoma, and has been linked with confidence to the plasmablastic variant of Castleman's disease, also called multicentric angiofollicular lymphoid hyperplasia. There are also a large number of reports on the PCR detection of HHV-8 DNA in a wide variety of diseases, including non-KS skin lesions in transplant recipients, reactive lymphadenopathy Bowen's disease, salivary gland tumours (bilateral mucosa-associated lymphoid tissue lymphoma of the parotid gland in HHV-8 seropositive patients), interstitial pneumonitis in HIV-positive individuals, persistent HHV-8 viraemia with co-infection with HTLV and myelofibrosis, angiosarcoma, pemphigus vulgaris in HIV-positive or HIV-negative patients and foliaceus, multiple myeloma, haemophagocytic syndrome, non-cleaved cell lymphoma, Kikuchi's disease and sarcoidosis (2, 15, 16).

HHV-8 has a limited and uneven geographic distribution. In Sub-Saharan Africa, antibodies to the virus can be found in 30% of the general population (15, 17). In the Mediterranean area, seropositivity may be 10–25%, with some geographical pockets in this area having higher or lower seropositivity (15). HHV-8 is prevalent in Greece in the range observed in other Mediterranean countries, and has been investigated recently in two studies. Zavitsanou et al. (18) tested for antibodies against lytic phase glycoprotein K8.1, using enzyme-linked immunoassay (ELISA) in healthy urban employees and the prevalence was found to be 7.6%. Zavos et al. (19) tested blood sera by nested PCR and found HHV-8 DNA in 9.6% of the healthy population. These results indicate that there is a remarkable presence of the virus in the general population in Greece. This fact is also indicated from the 0.47 new KS cases per 100,000 population per year, with KS representing 1.35% of all malignant neoplasms (20).

HHV-8 is able to infect a variety of human cells, such as PBMCs, bone marrow cells and keratinocytes (15). Cerimele et al. (21) found that HHV-8 can establish a

productive infection in primary human keratinocytes, and viral particles purified from the supernatant of infected keratinocytes can infect other primary cells, such as human endothelial cells. The virus can also be found in the peripheral blood monocytic cells of carriers, and the likelihood of finding such infected cells varies with the seroprevalence for a particular region. The time that virus remains in infected cells, varies according to the type of cell. In a study by Flore et al. (22), on primary endothelial cells, HHV-8 could always be detected in a subset of transformed primary bone marrow cells after culture for more than one year. However, in case of primary human keratinocytes, viral genomes were undetectable by nested PCR after 8 weeks, although the previously infected cells still displayed modified characteristics (21). This fact makes the detection of virus in lesional skin specimens very important, because it is declaring its presence due to primary infection or reactivation.

This is the first study to detect HHV-8 in the lesional skin of patients with PR. A former study of 8 patients by Chuh et al. (23), found no correlation between HHV-8 and PR. The aim of the present study was prove the implication of the virus in the pathogenesis of PR, and the positive sequencing of viral DNA is strong proof in this direction. The detection of HHV-8 does not exclude the presence of the other two viruses, HHV-6 and HHV-7, which appear to have an important role in PR pathogenesis. By contrast, the well-known interactions between herpesviruses pose a question about the mechanism of HHV-8 presence in lesional skin; whether it is the result of an individual process or a simultaneous action with HHV-6 and -7.

In conclusion, HHV-8 is a possible pathogenic factor in PR. Further studies are needed to determine the mechanism and extent of virus replication, correlation of viral DNA with antibody titres, and possible interaction with the HHV-6 and -7.

REFERENCES

1. Allen RA, Janniger CK, Schwartz RA. Pityriasis rosea. Cutis 1995; 56: 198–202.
2. Blauvelt A. Skin diseases associated with human herpesvirus 6, 7 and 8 infection. J Invest Dermatol Symp Proc 2001; 6: 197–202.
3. Drago F, Ranieri E, Malaguti F, Battifoglio ML, Losi E, Rebora A. Human herpesvirus 7 in patients with pityriasis rosea. Dermatology 1997; 195: 374–378.
4. Drago F, Ranieri E, Malaguti F, Losi E, Rebora A. Human herpesvirus 7 in pityriasis rosea. Lancet 1997; 349: 1367–1368.
5. Kempf W, Adams V, Kleinhans M. Pityriasis rosea is not associated with human herpesvirus 7. Arch Dermatol 1999; 135: 1070–1072.
6. Watanabe T, Kamamura T, Jacob SE, Aquilino E, Orenstein JM, Black JB, et al. Pityriasis rosea is associated with systemic active infection with both human herpesvirus-7 and human herpesvirus-6. J Invest Dermatol 2002; 119: 793–797.
7. Broccolo F, Drago F, Careddu AM, Foglieni C, Turbino L, Cocuzza CE, et al. Additional evidence that pityriasis rosea is associated with reactivation of human herpesvirus-6 and -7. J Invest Dermatol 2005; 124: 1234–1240.
8. Watanabe T, Sugaya M, Nakamura K, Tamaki K. Human herpesvirus 7 and pityriasis rosea (correspondence). J Invest Dermatol 1999; 113: 288–289.
9. Drago F, Rebora A. Pityriasis rosea: one virus, two viruses, more viruses? (correspondence). Br J Dermatol 2001; 144: 1086–1099.
10. Kosuge H, Tanaka-Taya K, Miyoshi H, Amo K, Harada R, Ebihara T, et al. Epidemiological study of human human herpesvirus 6 and human human herpesvirus 7 in pityriasis rosea. Br J Dermatol 2000; 143: 795–798.
11. Chuh AAT, Chiu SS, Peiris JS. Human herpesvirus 6 and 7 DNA in peripheral blood leucocytes and plasma in patients with pityriasis rosea by polymerase chain reaction: a prospective case control study. Acta Derm Venereol 2001; 81: 289–290.
12. Karabulut AA, Kocak M, Yilmaz N, Eksioglu M. Detection of human herpesvirus 7 in pityriasis rosea by nested PCR. Int J Dermatol 2002; 41: 563–567.
13. Drago F, Ranieri E, Rebora A. Pityriasis rosea and herpesvirus 7. Action or interaction? Dermatology 1998; 197: 275.
14. Drago F, Vecchio F, Rebora A. Use of high-dose acyclovir in pityriasis rosea. J Am Acad Dermatol 2006; 54: 82–85.
15. Ablashi DV, Chatlynne LG, Whitman Jr. JE, Cesarman E. Spectrum of Kaposi sarcoma-associated herpesvirus, or human herpesvirus 8, diseases. Clin Microbiol Rev 2002; 15: 439–464.
16. Plancoulaine S, Abel L, Gessain A. Epidemiology of human herpes virus 8 (HHV-8) or the herpes virus associated with Kaposi's sarcoma. Pathol Biol 2002; 50: 496–502.
17. Sarid R, Olsen SJ, Moore PS. Kaposi's sarcoma-associated herpesvirus: epidemiology, virology, and molecular biology. Adv Virus Res 1999; 52: 139–232.
18. Zavitsanou A, Sypsa V, Petrodaskalaki M, Kalapothaki V, Whitby D, Hatzakis A. Human herpesvirus 8 (HHV-8) infection in healthy urban employees from Greece: Seroprevalence and associated factors. J Med Virol 2007; 79: 591–596.
19. Zavos G, Gazouli M, Papaconstantinou I, Lukas JC, Zografidis A, Kostakis A, et al. Prevalence of human herpesvirus 8 DNA sequences in human immunodeficiency virus-negative individuals without Kaposi's sarcoma in Greece. In Vivo 2005; 19: 729–732.
20. Stratigos JD, Katoulis AC, Stavrianeas NG. An overview of classic Kaposi's sarcoma in Greece. Adv Exp Med Biol 1999; 455: 503–506.
21. Cerimele F, Curreli F, Ely S, Friedman-Kien AE, Cesarman E, Flore O. Kaposi's sarcoma-associated herpesvirus can productively infect primary human keratinocytes and alter their growth properties. J Virol 2001; 75: 2435–2443.
22. Flore O, Rafii S, Ely SJ, O'Leary JJ, Hyjek EM, Cesarman E. Transformation of primary human endothelial cells by Kaposi's sarcoma-associated herpesvirus. Nature 1998; 394: 588–592.
23. Chuh AAT, Chan PKS, Lee A. The detection of human herpesvirus-8 DNA in plasma and peripheral blood mononuclear cells in adult patients with pityriasis rosea by polymerase chain reaction. J Eur Acad Derm Venereol 2006; 20: 667–671.