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Original Article

Absence of Human Herpesvirus 8 in Pemphigus and Bullous Pemphigoid

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Abstract: Pemphigus and pemphigoid are vesicobullous disorders characterized by an autoimmune attack on intercellular or basement membrane antigens, resulting in defective keratinocyte adhesion. Recently there have been reports of human herpesvirus 8 (HHV8) associated with cases of pemphigus using polymerase chain reaction (PCR) techniques, in situ hybridization, and serologic data. However, data to date is contradictory, and the relationship between this virus and autoimmune vesiculobullous disorders is unclear. No reports have attempted immunohistochemical localization of HHV8 in tissue affected by PV or BP. We studied immunohistochemical expression of HHV8 on paraffin-embedded tissue in 10 cases of pemphigus vulgaris (PV), 1 case of pemphigus foliaceous (PF) and 14 cases of bullous pemphigoid (BP). Five cases of normal skin were included as controls. Confirmatory PCR for HHV8 was performed on 4 selected cases, including 2 cases of PV and 2 cases of BP. Immunohistochemistry failed to identified the presence of HHV8 in all cases of PV (10 cases), PF (1 case) and BP (14 cases). Molecular detection of HHV8 DNA was not detected in selected PV (2 cases) and BP (2 cases). Published studies have shown contradictory evidence regarding the presence of HHV8 in vesiculobullous diseases such as pemphigus and pemphigoid. Our results refute a causal relationship between HHV8 and PV, PF and BP.

Key Words: Pemphigus, bullous pemphigoid, human herpesvirus 8 (HHV8), immunohistochemistry, polymerase chain reaction

Introduction

Pemphigus and bullous pemphigoid (BP) are autoimmune disorders, characterized by vesiculobullous skin eruptions. The mechanism of these diseases has been linked to autoantibodies directed against different target antigens, resulting in a defect in the adhesion of keratinocytes.

The pemphigus group (PG) consists of several pathologic variants with pemphigus vulgaris (PV) and pemphigus foliaceus (PF) being the classic forms and accounting for approximately 90% of all cases. Clinically, PV presents in the 5th to 6th decades with painful erosions of the buccal mucosa and cutaneous fragile blisters. PF manifests as cutaneous scaly, crusted erosions on an erythematous base, often without mucosal involvement. Routine histological examination shows intraepithelial acantholysis with blister formation in the suprabasal area in PV and subcorneal in PF. The pathogenesis involves development of autoantibodies to desmogleins 1 and 3, leading to acantholysis and bullae formation [1 ,2, 3, 4].

Clinically, BP typically affects elderly patients, with symmetrical, predominantly flexural and intertriginous tense bullae. Histologically, there is a subepidermal blister with a variable number of eosinophils. BP is caused by antibodies against the two main transmembrane antigens, BPAg1 and BPAg2, associated with lamina lucida and the hemidesmosomes in basal keratinocytes [3, 5, 6, 7].

A diagnosis of pemphigus and/or pemphigoid can be definitively established by using the technique of direct (DIF) and indirect (IIF) immunofluorescence tests of the skin and serum, respectively, showing the characteristic patterns of immunoreactant deposition, specifically intercellular deposition within the

epidermis in pemphigus and a linear basement membrane pattern in BP.

Human herpesvirus-8 (HHV8) was first found in tissue from AIDS-associated Kaposi's sarcoma (KS). At the present time the virus has a strong link with the majority of cases of KS, including in HIV-seropositive and HIV-seronegative patients [8, 9, 10]. Detection of HHV8 DNA by polymerase chain reaction (PCR) of DNA in other skin lesions has been also reported, including Bowen's disease, squamous cell carcinoma, actinic keratoses, leukoplakia, Paget's disease, malignant melanoma, neurofibroma, chronic dermatitis and also normal skin [11]. Reports of an increased incidence of KS in patients with pemphigus and bullous pemphigoid without evidence of HIV infection [12, 13, 14, 15] prompted the search for direct associations of HHV8 and vesiculobullous disorders. In 1997, Memar *et al* reported HHV8 detection by PCR in tissue from four cases of PV and six cases of PF [16]. The same authors describe HHV8 in-situ hybridization (ISH) of some of PV lesions with HHV8 DNA localization in peri-lesional endothelial cells and basal keratinocytes (unpublished data). A few other molecular studies supported the original findings of HHV8 in pemphigus [17, 18, 19]. In contrast, other reports showed lack of HHV8 detection in vesiculobullous disorders [20, 21, 22, 23]. To the best of our knowledge, all previous studies were done by PCR, ISH and serology. We hypothesize that previous positive studies may be detecting HHV8 DNA within circulating monocytes contained in the biopsied skin, rather than in lesional tissue, therefore we sought to localize HHV8 by immunohistochemical (IHC) analysis in cases of PV, PF and BP.

Materials and Methods

Patients and Tissue

Twenty-five consecutive cases with confirmatory DIF studies, including ten cases of PV, one case of PF and fourteen cases of BP were identified and retrieved from the files of the Department of Dermatology at Yale University School of Medicine between 2003 and 2005. Two cases of BP were from the same patient. The original hematoxylin and eosin stained slides from formalin-fixed, paraffin-embedded skin biopsies were reviewed by two authors (JM and AG).

Immunohistochemistry

We examined IHC expression of HHV8 on all 25 cases, including 10 cases of PV, 1 case of PF and 14 cases of BP using a rat monoclonal antibody to latent nuclear antigen (LNA-1, ORF-73) of HHV8. The protocol was followed according to the manufacturer's instructions (LN53, ABI, Columbia, MD, USA). Specimens of Kaposi's sarcoma were used as positive controls. Five cases of normal skin were used as negative controls.

PCR Studies

Four cases, including two of PV and two of BP were studied with PCR for HHV8. Ten 10-μm-thick sections were cut on the microtome for each case. After deparaffinization, DNA was extracted by proteinase-K digestion followed by standard extraction procedures using Qiagen DNA tissue kit (Qiagen, Chatsworth, CA) according to the manufacturer's instruction. The concentration of DNA preparation was determined by its absorbance at 260 nm. PCR, using specific primers for 268-bp-long fragment for beta-globin (verification of amplifiable DNA) and 233-bp-long fragment for HHV8 (ORF26) were used to assess the presence of HHV8 in all cases. The protocol and primer sets were followed as formerly described [9].

Results

Table 1 summarizes the clinical data and results of the HHV8 IHC and PCR studies. The mean age in the pemphigus group was 62.9 years old, ranging from 37 to 99 years old. The male to female ratio was 1:10 and the lesions were located on the upper and lower extremities, and trunk. Histologically, PV lesions displayed a range of features, including edema, loss of intercellular bridges and acantholysis, and well developed suprabasal bullae with "tombstoning" and occasional neutrophils and eosinophils. The PF lesion showed subcorneal separation with a few acantholytic keratinocytes present. The mean age in BP group was 72.9 years old, ranging from 56 to 86 years old. The male to female ratio was 3:10, and the lesions were located on the upper and lower extremities, trunk and neck. Microscopically, the lesions were characterized by subepidermal blisters, and eosinophils were the principal inflammatory cell in the blister cavity and

Table 1 Summary of the clinical data and results of IHC and PCR studies

Case number	Bullous disorder	Age (years)	Gender	Location	HHV8 IHC results	HHV8 PCR results
1	PV	61	Female	Forearm	(-)	NP
2	PV	39	Female	Back	(-)	NP
3	PV	99	Female	Abdomen	(-)	NP
4	PV	57	Female	Back	(-)	(-)
5	PV	37	Female	Leg	(-)	(-)
6	PV	56	Female	Axilla	(-)	NP
7	PV	79	Female	Back	(-)	NP
8	PV	68	Female	Leg	(-)	NP
9	PV	73	Female	Arm	(-)	NP
10	PV	45	Male	Shoulder	(-)	NP
11	PF	78	Female	Back	(-)	NP
12	BP	69	Female	Arm	(-)	NP
13	BP	86	Female	Arm	(-)	(-)
14	BP	84	Female	Arm	(-)	NP
15	BP	78	Male	Thigh	(-)	NP
16	BP	84	Male	Neck	(-)	NP
17	BP	86	Female	Arm	(-)	NP
18	BP	84	Female	Hand	(-)	(-)
19	BP	72	Male	Back	(-)	NP
20	BP	85	Female	Chest	(-)	NP
21*	BP	56	Female	Forearm	(-)	NP
22*	BP	56	Female	Hand	(-)	NP
23	BP	81	Female	Leg	(-)	NP
24	BP	85	Female	Arm	(-)	NP
25	BP	79	Female	Thigh	(-)	NP

NP, not performed; *, same patient; BP, bullous pemphigoid; PV, pemphigus vulgaris; PF, pemphigus foliaceus

dermis. Since our study was retrospective, there was no serological data available regarding HHV8 specific IgG antibodies.

IHC for HHV8 was negative in all tested cases, including 10 cases of PV, 1 case of PF, 14 cases of BP, as well as 5 negative control cases (see representative H&E and HHV8 IHC samples of PV, PF and BP in **Figure 1**).

The PCR-based analysis did not reveal HHV8 DNA in 4 representative cases, including 2 cases of PV and 2 cases of PF (see **Figure 2**). Due to tissue availability, we were not able to perform PCR on all samples.

Discussion

Pemphigus and bullous pemphigoid are autoimmune mediated blistering diseases. The pathogenesis of PV and PF includes anti-desmoglein 1 and 3 antibodies, the development of which may be mediated by genetic and environmental factors. Defects in other adhesion proteins, such as P-cadherin and focal adhesion kinase $p125^{fak}$, have also been identified. Abnormalities of the complement system, cytokines, and cell and humoral mediated immune responses have also been implicated in the development of PV. Other factors, such as drugs, trauma, surgery, ionizing radiation, thermal burns, PUVA treatments, chemicals, and foods have been described to precipitate an outbreak of pemphigus [24].

BP comprises approximately 80% of subepidermal autoimmune bullous disorders. The mechanism involves autoantibodies directed against antigens in the lamina lucida and hemidesmosomes of basal keratinocytes, known as BPAg1 (a.k.a. BP230) and BPAg2 (a.k.a. BP180). BPAg1 belongs to the plakin family and is restricted to the intracellular hemidesmosomal plaque, whereas the BPAg2 is a transmembrane glycoprotein with intracellular and extracellular interactions with hemidesmosomes. The most common immunoglobulin overexpression is of IgG_4, IgG_1, IgE and less frequently of IgA types. Other rare antigens have been reported, such as 105kd, 120kd, 190kd 240kd, 138kd, plectin and desmoplakin. Immune complex formation activates the complement system leading to

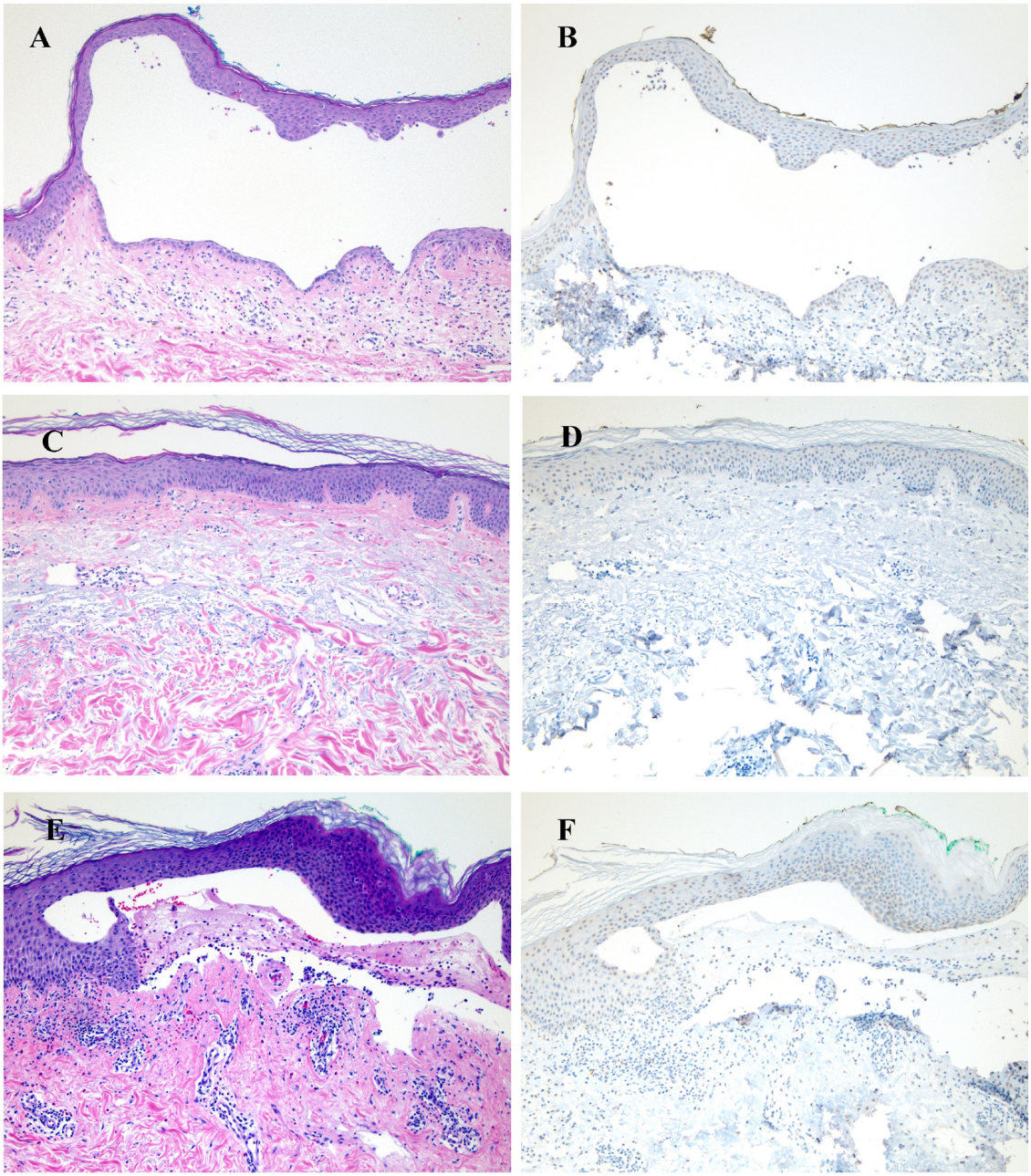

Figure 1 Representative histological images of PV, PF and BP lesions (**A**, **C** and **E**, respectively, H&E stain, 10x) with their corresponding negative HHV8 immunohistochemistry (**B**, **D** and **F**, respectively, 10x).

cellular chemotaxis and release of proteolytic enzymes, which contribute to the subepidermal blister formation. The cause of antibody formation is not clear, and has been linked to drugs, trauma, burns, phototherapy and radiation [24].

HHV8 was originally identified in AIDS-associated KS and subsequently has been demonstrated in as many as 100% KS of non-HIV patients [8-10]. The identification of KS in patients with pemphigus and bullous pemphigoid without evidence of HIV infection [12-15] has prompted studies in search of a direct relationship between HHV8 and vesiculobullous disorders. In our review of the literature, we found several studies done by PCR, ISH and serology with contradictory results (see **Table 2**). There are no previous reports on IHC method used for HHV8

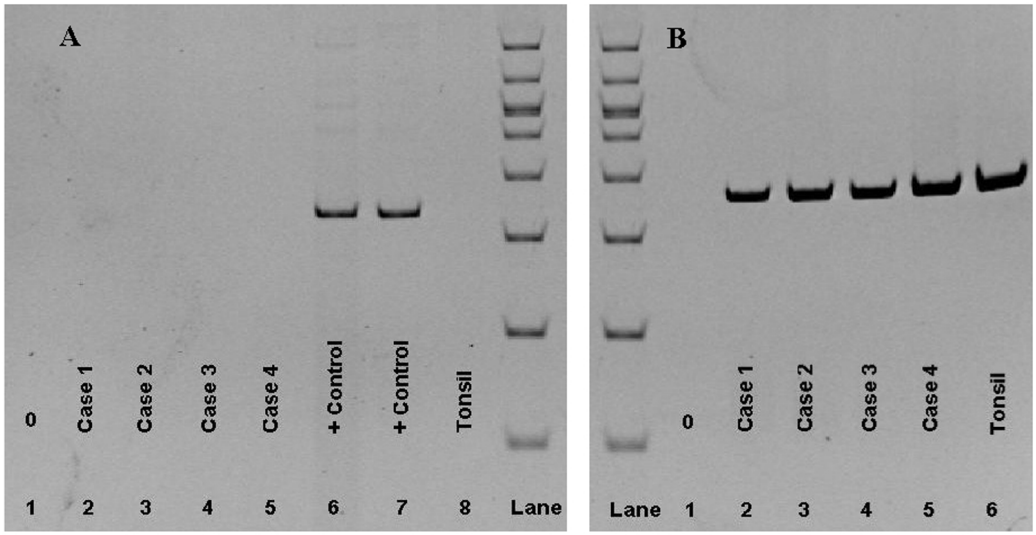

Figure 2 PCR analysis of HHV8 DNA in representative PV and BP. **A.** Absence of HHV8-specific PCR product by gel electrophoresis in 2 cases of PV and 2 cases of BP (lanes 2 to 5), along with positive (lanes 6 and 7) and negative (lanes 1 and 8) controls. **B.** PCR amplification of β-globin gene confirming the presence of amplifiable DNA extracted from the cases (lanes 2 to 5) and positive control (lane 6).

localization in pemphigus and BP. We used an immunohistochemical method to evaluate the presence of HHV8 in lesions of pemphigus and BP, since it is routinely employed to detect HHV8 in KS cases. Our study established no expression of HHV8 in PV, PF and BP. Therefore, our findings contradict a causative role for HHV8 in pemphigus and BP, and support other data refuting this possibility.

Table 2 Review of the literature regarding the relationship of vesiculobullous diseases and HHV8

Vesiculobullous disorder/material	Number of cases	Method	Number of positive cases	Number of negative cases	Reference
PV, skin lesion	1	PCR	1	0	15
PF, skin lesions	6	PCR	6	0	16
PV, skin lesions	6	PCR	4	2	
PV, skin lesions	9	PCR	7	2	17
PF, skin lesions	2	PCR	1	1	
PV, skin lesions	36	PCR	13	23	18
PV, PBMCs	13	PCR	4	9	
PV, serum	29	Serology	10	19	
PV, serum	9	Serology	1	8	19
BP, serum	11	Serology	1	10	
PV, skin lesions	10	ISH	0	10	20
PV, skin lesions	5	PCR	0	5	
PF, skin lesions	5	PCR	0	5	
PV, serum	19	Serology	0	19	
PV, serum	16	Serology	0	16	21
PF, serum	7	Serology	0	7	
PV, skin lesions	15	PCR	0	15	22
PBMCs	15	Serology	0	15	
PF, skin lesions	2	PCR	0	2	
PBMCs	2	Serology	0	2	
PV, skin lesions	6	PCR	0	6	23
PF, skin lesions	3	PCR	0	3	

PBMCs, Peripheral blood mononuclear cells; BP, bullous pemphigoid; PV, pemphigus vulgaris; PF, pemphigus foliaceus; ISH, in situ hybridization; PCR, polymerase chain reaction

The reports of HHV8 DNA in patients with vesiculobullous disorders may be due to the presence of bystander viral particles, which may be detected by highly sensitive methods, such as PCR, ISH and serology. HHV8 is likely a ubiquitous virus that infects the general population worldwide and is found at various rates of prevalence. As reported, HHV8 may be harbored by peripheral blood mononuclear cells in up to 23.8% of healthy Italian individuals and the incidence of KS may be directly proportional to the incidence of HHV8 seropositivity [10]. The rate of HHV8 seropositivity in other general populations ranges from 0.2% to 2% in French and Japanese studies to over 20% in the United States reports [14, 21, 25]. The significance of latent HHV8 infection is not fully understood yet. Most patients with pemphigus or BP are on immunosuppressive treatment, which may allow viral reactivation, therefore resulting in a higher detection rate of HHV8 DNA sequence and specific IgG antibodies in tissue and serum in this population [18]. As obligate intracellular life forms, viruses depend on host cell metabolism for their survival. Some viruses remain in lifelong latency, only to be reactivated with changes in the immune status of the host, which may be the case in vesicobullous disorders. However, we did not found HHV8 present in the lesions of PV, PF and BP by IHC and PCR, refuting a direct causal role for this virus within cutaneous lesions of these autoimmune blistering disorders. We hypothesize that circulating monocytes harboring HHV8 may be the source of reported positive PCR results for HHV8 in patients with BP and PV.

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References

[1] Hashimoto T, Ogawa MM, Konohana A and Nishikawa T. Detection of pemphigus vulgaris and pemphigus foliaceous antigens by immunoblot analysis using different antigen sources. *J Invest Dermatol* 1990;94:327-331.

[2] Amagai M, Tsunoda K, Zillikens D, Nagai T and NishikawaT. The clinical phenotype of pemphigus is defined by the anti-desmoglein autoantibody profile. *J Am Acad Dermatol* 1999;40:167-170.

[3] Bolognia JL, Jorizzo JL and Rapini RP. In: Dermatology. Mosby 2003;vol 1:pp449-477.

[4] Korman N. Pemphigus. *J Am Acad Dermatol* 1998;18:1219-1238.

[5] Korman NJ. In situ-bound antibodies eluted from the skin of patients with bullous pemphigoid are preferably directed against the 230-kD bullous pemphigoid antigen. *J Invest Dermatol* 1995;105:824-830.

[6] Hopkinson SB, Ridelle KS and Jones JCR. Cytoplasmic domain of the 180-kD bullous pemphigoid antigen a hemidesmosomal component: molecular and cell biologic characterization. *J Invest Dermatol* 1992;99:243-250.

[7] Farmer ER. Subepidermal bullous diseases. *J Cutan Pathol* 1985;12:316-321.

[8] Chang Y, Cesarman E, Pessin MS, Lee F, Culpepper J, Knowles DM and Moore P. Identification of herpes virus-like DNA sequence in AIDS-associated Kaposi's sarcoma. *Science* 1994;266:1865-1869.

[9] Kazakov DV, Prinz BM, Michaelis S, Schmid M, Müller B, Adams V, Burg G and Kempf W. Study of HHV-8 DNA sequences in archival biopsies from lesional skin of Kaposi's sarcoma, various mesenchymal tumors and reactive conditions. *J Cutan Pathol* 2002;29:279.

[10] Cattani P, Capuano M, Lesnoni La Parola I, Guido R, Santangelo R, Cerimele F, Masini C, Nanni G, Fadda G and Cerimele D. Human herpesvirus 8 in Italian HIV-seronegative patients with Kaposi sarcoma. *Arch Dermatol* 1998;134:695-699.

[11] Nishimoto S, Inagi R, Yamanishi K, Hosokawa K, Kakibuchi M and Yoshikawa K. Prevalence of human herpesvirus-8 in skin lesions. *Br J Dermatol* 1997;137:179-184.

[12] Younus J and Ahmed AR. The relationship of pemphigus to neoplasia. *J Am Acad Dermatol* 1990;23:498-502.

[13] Halpern SM, Parslew R, Cerio R, Kirby JTD and Sharpe GR. Kaposi's sarcoma associated with immunosuppression for bullous pemphigoid. *Br J Dermatol* 1997;137:140-143.

[14] Sato-Matsumura KC, Matsumura T, Nabeshima M, Katano H, Sata T and Koizumi H. Serological and immunohistochemical detection of human herpesvirus 8 in Kaposi's sarcoma after immunosuppressive therapy for bullous pemphigoid. *Br J Dermatol* 2001;145:633-637.

[15] Memar OM, Rady PL, Goldblum RM and Tyring SK. Human herpesvirus-8 DNA sequence in a patient with pemphigus vulgaris, but without HIV infection or Kaposi's sarcoma. *J Invest Dermatol* 1997;108:118-119.

[16] Memar OM, Rady PL, Goldblum RM, Yen A and Tyring SK. Human herpesvirus 8 DNA sequences in blistering skin from patients with pemphigus. *Arch Dermatol* 1997;133:1247-1251.

[17] Jang HS, Oh CK, Lim JY, Jun ES, Kim YS and

Kwon KS. Detection of human herpesvirus 8 DNA in pemphigus and chronic blistering skin diseases. *J Korean Med Sci* 2000;15:442-448.
[18] Wang GQ, Xu H, Wang YK, Gao XH, Zhao Y, He C, Inoue N and Chen HD. Higher prevalence of human herpesvirus 8 DNA sequence and specific IgG antibodies in patients with pemphigus in China. *J Am Acad Dermatol* 2005;52:460-467.
[19] Dupin N, Marcelin AG, Gorin I, Bossi P, Franck N, Weill B, Huraux JM, Escande JP and Calvez V. Prevalence of human herpesvirus 8 infection measured by antibodies to a latent nuclear antigen in patients with various dermatologic diseases. *Arch Dermatol* 1998;134:700-702.
[20] Cohen SS, Weinstein MD, Herndier BG, Anhalt GJ and Blauvelt A. No evidence of human herpesvirus 8 infection in patients with paraneoplastic pemphigus, pemphigus vulgaris, or pemphigus foliaceous. *J Invest Dermatol* 1998;111:781-783.
[21] Dupin N, Marcelin AG, Calvez V and Andre C. Absence of a link between human herpesvirus 8 and pemphigus. *Br J Dermatol* 1999;141:159-160.
[22] Tufano MA, Baroni A, Buommino E, Ruocco E, Lombardi ML and Ruocco V. Detection of herpesvirus DNA in peripheral blood mononuclear cells and skin lesions of patients with pemphigus by polymerase chain reaction. *Br J Dermatol* 1999;141:1033-1039.
[23] Bezold G, Sander CA, Flaig MJ, Peter RU and Messer G. Lack of detection of human herpesvirus (HHV)-8 DNA in lesional skin of German pemphigus vulgaris and pemphigus foliaceous patients. *J Invest Dermatol* 2000;114:739-741.
[24] Weedon D. Skin Pathology (2nd Ed). Churchill Livingstone, 2002, pp129-91.
[25] Lennette E, Blackbourn D and Levy JA. Antibodies to human herpesvirus type 8 in general population and in Kaposi's sarcoma patients. *Lancet* 1996;348:858-861.