Case Report

Junctional adhesion molecule overexpression in Kaposi varicelliform eruption skin lesions - as a possible herpes virus entry site

Ana Maria Abreu-Velez[1], MD., PhD, A. Deo Klein[2], III, MD., Michael S. Howard[1], MD.

[1]Georgia Dermatopathology Associates, Atlanta, Georgia, USA.
[2] Statesboro Dermatology, Statesboro, Georgia, USA.

Abstract

Context: Herpes simplex virus (HSV) infection of the skin represents a common challenge in dermatology; however, currently the port of viral entry remains obscure. HSV is known to induce an immunoglobulin-binding cell surface receptor in infected cells that utilizes a non-immune mechanism. The replication of HSV in cultured cells is accompanied by the appearance of surface receptors with an affinity for the Fc region of immunoglobulin G. **Case Report**: We describe a 43 year old African American male who presented with a generalized rash, including intense pruritus and umbilicated vesiculopustules. The patient had been previously diagnosed and treated for psoriasis with methotrexate and prednisone. **Methods**: Hematoxylin and eosin demonstrated keratinocytes with ballooning degeneration within the epidermis. Direct immunofluorescence (DIF) results resembled the pattern of paraneoplastic pemphigus, with negative indirect immunofluorescence (IIF) results on rat bladder. Immunohistochemistry revealed deposits of the complement membrane attack complex within dermal sweat glands, as well as the presence of herpes simplex virus 1 on the skin. We report a case of Kaposi varicelliform eruption, a cutaneous eruption caused by a virus infecting patients with pre-existing dermatoses. **Conclusion:** HSV virus infection with over-expression of the junctional adhesion molecule close to herpetic infection sites may preferentially increase viral entry through the skin, possibly triggering a Kaposi varicelliform eruption.

Keywords: Kaposi varicelliform eruption, herpes immunoglobulin receptor, herpes simplex virus, junctional adhesion molecule A, sweat glands, cell junctions, melanophages, nerves.

Correspondence to: Ana Maria Abreu-Velez, MD, PhD, Georgia Dermatopathology Associates, 1534 North Decatur Rd. NE; Suite 206, Atlanta, GA 30307-1000, USA. Tel.: (404) 371 0027, Fax: (404) 371 1900, Email: abreuvelez@yahoo.com

Introduction

Historically, the classification of blistering disorders has been attempted by clinical features (i.e., presence of vesicles, bullae), etiologic factors (i.e., infectious, immunologic), or by histologic parameters (i.e., intraepidermal, subepidermal) [1]. Each of these classification systems confers advantages; there remains, however, 1) a small number of clinical conditions that rarely manifest as a blistering disorder or 2) uncommon conditions that routinely blister, thus defying conventional classification. Such cases comprise a miscellaneous group of blistering conditions worthy of special consideration, in which the diagnosis may not be easily achieved [1].

Herpes virus infection lies within the differential diagnoses of blistering skin diseases. In order to achieve pathogenic infection, the virus must breach the epithelial barriers that either separates the organism from the external environment, or that cover the internal cavities and ducts of the body. Epithelia may allow viral passage via the paracellular pathway, with the apical junctional complex (AJC) including tight and adherens junctions [2, 3]. In this case, we describe how viruses, including herpes simplex 1, may potentially utilize an overexpression of intercellular junction receptors present at the AJC of epithelial cells [2, 3].

Case Report

We describe a 43 year old African American male who presented with a generalized rash of sudden onset, with intense pruritus and umbilicated vesiculopustules over the entire body. The patient had been treated for previously diagnosed psoriasis with methotrexate and prednisone. The eruption continued to spread for 7-10 days and was associated with high temperature, malaise, and lymphadenopathy. Two punch biopsies were taken, with one placed into Michel's transport medium (Newcomer Supply Laboratories, Middleton, Wisconsin, USA) and the second into 10% buffered formalin.

Hematoxylin and eosin (H&E)
Tissue examination demonstrated multilevel blistering of the epidermis, with some re-epithelialization of the blister base also present (Fig. 1). Isolated areas of the epidermis displayed suprabasal blistering. In addition, focal areas of the epidermis displayed ballooning of keratinocytes, with margination of cell chromatin present and occasional multinucleated cells seen. Eosinophilic inclusions were noted within selected keratinocytes. Within the blister lumen, numerous neutrophils and occasional eosinophils were present. No evidence of a neoplastic process was seen. The dermis displayed a mild, superficial, perivascular infiltrate of lymphocytes, histiocytes and occasional neutrophils and eosinophils.
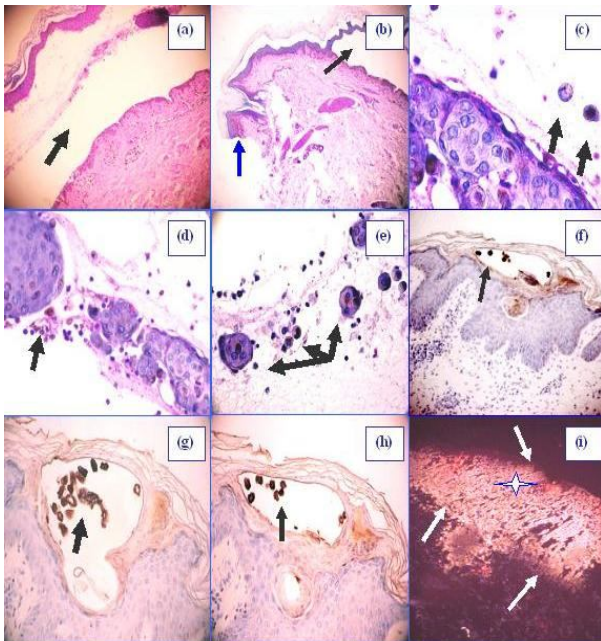

Figure 1 a. H & E, showing a subepidermal blister (black arrow). **b.** PAS shows also subepidermal blister (black arrow), and some base membrane reinforcement (blue arrow). **2c,d** and **e**, Some herpetical ballooning of keratinocytes, with margination of cell chromatin present and occasional multinucleated cells with cytopathic effects(black arrows) **f, g**, and **h** IHC positive staining to HSV 1(black arrows). **i**. DIF positive staining with anti-JAM-A antibody (pink staining, indicated with white arrows and star) in the entire epithelial area where the HSV virus was found.

Direct immunofluorescence (DIF)
For DIF, four micron thick skin cryosections were prefixed in paraformaldehyde, rinsed in phosphate-buffered saline (PBS, pH 6.8) and incubated with fluorescein isothiocyanate (FITC) in a solubilization buffer (PBS with 0.5% Triton X-100), and then rinsed. After blocking with PBS including 0.01%-Tween 20 (polysorbate 20) and 0.5% bovine serum albumin, the sections were incubated with antisera for one hour. We used FITC-conjugated rabbit antisera against fibrinogen and albumin at a 1:40 dilution. We also utilized FITC-conjugated rabbit anti-human IgG (gamma chain) and IgA (alpha chain) antisera (both 1:20 dilutions) and FITC conjugated rabbit anti-human IgM (Mu-chain) antiserum, all purchased from Dako (Carpinteria, California, USA). We also used goat anti-human IgE (epsilon-chain) antiserum, conjugated anti-junctional adhesion molecule (JAM-A) antibody (Invitrogen, Carlsbad, California, USA) (recognizing type I transmembrane glycoproteins of the immunoglobulin superfamily localized within tight junctions). As a secondary antibody, we utilized Texas red-conjugated sheep anti-mouse IgG (H & L) antiserum (Rockland Immunochemicals, Inc., Gilbertsville, Pennsylvania, USA) at 1:100 dilution. We used mouse anti-ezrin antibodies conjugated with Alexa Fluor® 647 (Invitrogen). We also used Cy3 conjugated anti-human glial fibrillary acidic protein(GFAP) (Sigma-Aldrich, Saint Louis, Missouri, USA).Finally, the sections were examined with a Nikon Eclipse 50i microscope (Tokyo, Japan) using a Xenon arc light (XBO 75W) as the light source and a plane achromatic (PL Apo) × 40/0.80 dry objective. The fluorescent staining was graded as follows: − (negative), ± (doubtful), + (weak), ++ (moderate), and +++ (bright). In Figure 2, we show several DIF findings, including positive staining for JAM-A in the area of the blister. In addition, we demonstrate strong staining with ezrin and positive intercellular staining (ICS) between the basal keratinocytes, simultaneously with staining against the basement membrane zone (BMZ) of the skin.
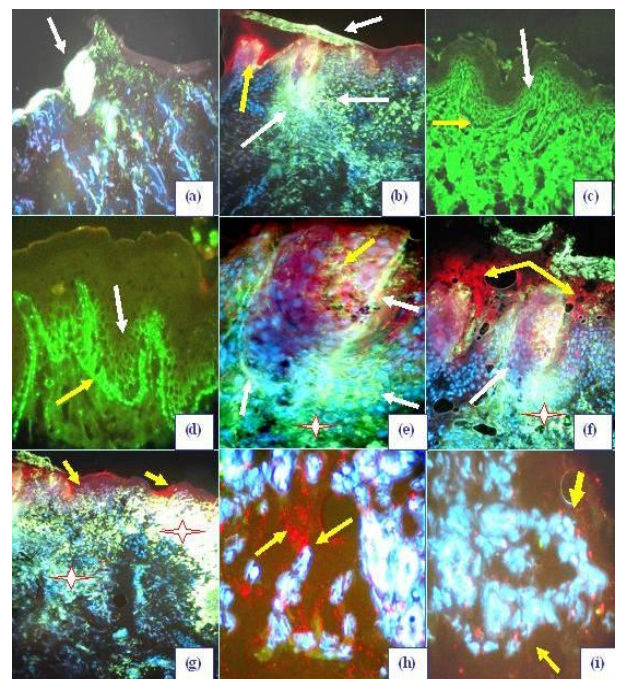

Fig. 2 All DIF. 2a, positive staining for JAM-A in the area of the blister (white staining, white arrow). **2b,** positive staining against JAM-A in several areas of the skin (white staining, white arrows).

The yellow arrow shows positive staining against ezrin. **2c.** Positive intercellular staining (ICS) between the basal keratinocytes (white arrow), and against the basement membrane zone (BMZ) of the skin (yellow arrow) using FITC conjugated anti-human IgG, possibly as results of the affinity of the herpes virus for the Fc portion of the immunoglobulins. ELISA studies for desmogleins 1 and 2 were both negative, as well as the immunoblotting on skin extracts. **2d** Similar to **2c,** but in this case with Complement/C3.**2 e, f** and **g,** Show positive staining in red for ezrin (yellow arrows), against JAM-A (diffuse areas in white), (stars), and against FITC conjugated human IgG (greenish staining, white arrows). **h,** and **i,** Positive staining against ezrin (red staining, yellow arrows). The nuclei were counterstained with DAPI (blue).

Immunohistochemistry studies (IHC)
To study correlation of the herpes virus infection with our JAM-A and ezrin DIF data, we performed IHC utilizing a Dako dual endogenous peroxidase blockage system, with the addition of an Envision dual link. We followed Dako technical instructions for staining. We applied 3, 3 diaminobenzidine (DAB) as an IHC chromogen, and counterstained with hematoxylin. We tested for varizella zoster antibody (Santa Cruz Biotechnology, Santa Cruz, California, USA), as well as for herpes virus 1 and 2(both from Dako). Immunoblotting and ELISA were performed as previously described [6, 7].

Figure 3 highlights our IHC results, including the compartmentalization of vimentin around several skin appendices where the DIF autoreactivty was detected. The degree of compartmentalization of vimentin by IHC parallels the deposition of immunoglobulins, complement and other immunosurfactants (such as fibrinogen) by DIF.
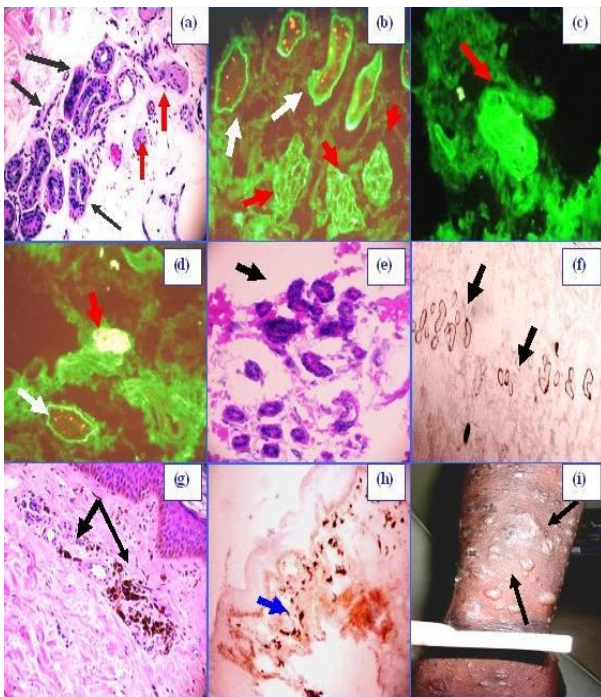

Fig. 3 (DIF in b, c and d, H& E in a, e, g and bleached H& E in h, IHC in f). **a,** Some alterations in dermal sweat glands (black arrows), and their respective nerves (red arrows), under the area where the viral infection was detected in the epidermis. **b**. Positive staining of the sweat glands (white arrows, green

staining) and the nerves (red arrows, green staining with FITC conjugated anti human IgG and C3 -FITC antibodies). **c**. The positive nerve, at higher magnification (green staining, red arrow). **d**. Similar to **b** and **c**, but in this case, to confirm the identity of the nerve, we co-localized the nerve by staining with Cy3 conjugated anti-human glial fibrillary acidic protein (GFAP)(white staining, red arrow). A nearby sweat gland is indicated by the white arrow. **e**. Some rarefaction of the sweat glands on H&E at higher magnification. **f.** IHC positive staining of Complement/C5-C9/MAC on sweat glands (brown staining; black arrow). **g** and **h**. Melanin bleaching confirmed the presence of melanin in those pigmented areas (H&E and melanin bleaching, respectively; black arrows). **i** Shows the clinical generalized vesicles and bullae on the upper arm (black arrow).

Discussion

A significant challenge in dermatology practice concerns the diagnosis and treatment of blistering diseases [1, 4-6]. Helpful clinical information includes 1) the patient's age and time of lesion onset; 2) the size, incidence and location of blisters; 3) possible inciting factors (including trauma, sun exposure, foods, drugs, infections, and neoplasms); 4) prior diagnostic attempts and therapies, and 5) extent of pain or pruritis [1, 4-6]. Review of systems should include a review of alterations in growth or development, and mucosal involvement (including any oral, nasopharyngeal, ocular, genitourinary, gastrointestinal or respiratory symptoms). Any family history of blistering diseases and any relevant geographic area of onset or ethnic ancestry should also be evaluated [1, 4-6]. A complete physical examination should then be performed, highlighted by inspection of skin and mucosal surfaces. Blister size, location and character should be evaluated, including an estimate of the anatomic split level of the blisters. Superficial blisters often manifest as crusted erosions; intraepidermal blisters are often flaccid; and may expand under pressure. Intra-lamina lucida blisters are often tense and heal with no scarring, but occasionally atrophy. Sub-lamina densa blisters often heal with scarring, milia are often present. Any nail, hair, or dental involvement should be documented. [1, 4-6].

In our patient, although we found some clinical suggestions of herpes virus involvement, we needed laboratory confirmation of this possibility. We utilized IHC staining for HSV 1, since both varizella zoster and herpes simplex viruses present similar viral cytopathic effects on H & E examination.

In the diagnosis of cutaneous blistering diseases on the skin, clinical or histological findings alone may not provide an accurate diagnosis. After the history, physical examination and H&E histologic examination, DIF examination is also warranted [1, 4-7]. In selected cases, additional specialized laboratory tests should be obtained. For example, if a porphyria is suspected, urine, blood and/or stool porphyrin levels may be helpful, depending on the porphyria subtype; if bullous systemic lupus erythematosus is suspected, antinuclear antibodies, double-stranded DNA antibodies or other serologic tests may be informative. DIF of a perilesional biopsy (near but not including a fresh blister) will often yield the most

informative results. Indirect immunofluorescence (IIF) may be also performed, with 0.1 M sodium chloride (NaCl) salt split skin or diastase, to ascertain an accurate intra-lamina lucida or dermal-epidermal blister separation level. The advantage of the salt split skin technique is that the location of any antibody/complement deposits can be quickly determined, especially in cases where bullous pemphigoid (BP) or epidermolysis bullosa aquisita (EBA) is suspected. In EBA, the pathogenic autoantibodies are classically found on the blister floor, in contradistinction to BP, where the pathogenic antibodies are found classically on the blister roof, and uncommonly on both the blister roof and floor [1, 4-7]. While the salt split skin IIF technique is most often used for differentiating BP from EBA, the technique may also be used to diagnose less common autoimmune diseases. For example, it is useful in the analysis of cicatricial pemphigoid sera, in that a subset of patient sera contain autoantibodies localizing to the blister floor, whereas a subset of patient sera contain autoantibodies localizing to the blister roof [1, 4-7]. Immunoblotting utilizing skin extracts may help to further confirm specific autoimmune diseases, including selected variants of pemphigus and bullous pemphigoid [1, 4-7]. Immunoelectron microscopy may also prove of significant assistance. In addition, enzyme-linked immunosorbent assay (ELISA) testing may be helpful in detecting autoantibodies to pemphigus and pemphigoid; ELISA tests are commercially available to confirm the presence of molecules such as desmogleins 1 and 2, BP180, BP230, type VII collagen, and laminin-5.

In our case, the differential diagnosis included several autoimmune blistering diseases, especially paraneoplastic pemphigus (PNP) due to immunoreactivity noted simultaneously between epidermal keratinocytes, as well as against the epidermal basement membrane zone. However, our patient serum did not react with rat bladder, and did not test positive for plakins or desmogleins by immunoblotting testing, as routinely seen in patients with PNP. The other differential diagnosis we disproved was pemphigus erythematosus, also known as Senear-Usher syndrome, an overlap syndrome with features of lupus erythematosus (LE) and pemphigus foliaceus. Pemphigus is demonstrated by acantholysis and immunoglobulin deposits in the interkeratinocyte spaces. The lupus component of pemphigus erythematosus is demonstrated by circulating antinuclear antibodies (ANA) and sometimes by immunoglobulin and complement deposits at the dermoepidermal junction. In this case, our ANA tests were negative, and the clinical lesions were not only distributed in the seborreic areas, but included generalized and flattened blisters. In addition, our patient displayed no superficially eroded lesions in sun exposed areas, such as the face or the upper part of the chest. Of interest, we did confirm the presence of HSV virus by IHC staining. We also ruled out diseases (including pemphigus and pemphigoid) utilizing ELISA testing and immunoblotting. Utilizing DIF, we were able to observe autoantibody intercellular staining between keratinocytes, as well as some basement membrane staining. Reviewing the indexed literature, we were not able to find documentation of this specific immune response pattern in cases of a Kaposi varicelliform eruption (KVE) [8, 9]. We can thus only speculate about our DIF findings, knowing that HSV infects many cell types. No clinical evidence of a neoplastic process was present in our patient.

The major target cells during primary and recurrent HSV infections are cells of epithelial and neural origin. In addition, the most clearly delineated risk factor for KVE is disruption of the epidermal barrier. During initial exposure, HSV infects epithelial cells such as epidermal keratinocytes, and spreads through the epithelium [10-13]. Virions next infect the axonal terminals of sensory neurons that innervate the superficial dermis. HSV then travels by retrograde axonal transport to the neuronal cell body. The virus may then abandon the replicative process, and establish a latent infection. Following episodic reactivation, newly replicated HSV is transported back to the axonal terminal regions and spreads to infect epithelial cells, often leading to a recurrent herpetic lesion. [11-13]. Further, replication of HSV in cultured cells is accompanied by the appearance of receptors that have an affinity for the Fc region of immunoglobulin G (IgG) [10-13]. The first indication of the existence of these receptors came from experiments that demonstrated the acquired capacity of HSV-infected cells to bind to antibody-coated erythrocytes [10-13]. In addition, it has been shown that cells infected with either HSV types 1 or 2 (HSV-1 or 2) could bind to free IgG or antigen-associated IgG from several species; the binding was to the Fc regions of the IgG molecules [10-13]. Thus, we speculate that JAM-A overexpression within herpetic skin lesions could play a role in herpes virus entry through the herpes immunoglobulin receptor; and moreover, that this molecule could be associated with the previously discussed affinity for the Fc portion of the IgG molecules on infected keratinocytes.

We also observed something commonly seen in autoimmune blistering diseases, that is, the histologic presence of dermal melanophages [6-7]. Viruses are thought not to use unique viral receptors, but to associate instead with a variety of host cell molecules. Thus, viruses mimic the natural ligands of these cell receptors and interfere with their signaling to promote viral entry into the organism. Virus to cell attachment is a multi-step process that involves recognition and communication by various proteins with variable sequences, structures and cellular functions [10-13]. Important viral attachment receptors include 1) carbohydrate chains of proteoglycans and epithelial membrane glycosphingolipids that bind to viral surface proteins [10-13]; and 2) integrins concentrated at the basolateral surface of epithelial cells, where they mediate interaction with molecules of the extracellular matrix; the matrix molecules exhibit specific motifs, which are also found in viral surface proteins [10-13]; and 3) cell-cell adhesion molecules, including proteins present at adherens junctions and tight junctions [11-13]. We speculate that the cell-cell adhesion molecule interactions may occur following damage to the basement membrane zone, with accompanying melanin pigment incontinence and a subsequent increase in pigmented

melanophages in the superficial dermis. Of interest, our findings of JAM-A overexpression on selected cell junctions have also been noted in viral infections by other authors. Specifically, multiple viruses including coxsackie virus, swine vesicular disease virus, adenovirus, reovirus, feline calcivirus, herpes viruses 1 and 2, pseudorabies virus, bovine herpes virus 1, poliovirus and hepatitis C virus utilize integral proteins present at the AJC of epithelial cells as cellular receptors [2,3,10-13]. Similarly, in our case we were able to see some overexpression of JAM-A and ezrin around the areas where the virus was histologically detected within the skin. We were also able to see disease autoreactivity by DIF in the areas of the skin where cells junctions are located, i.e., between epidermal keratinocytes and at the basement membrane zone. Viral surface proteins contribute significantly to defining the unique tropism of each virus. Besides these proteins, viruses exhibit a wide range of cellular co-receptors, among which integrin proteins are found; these viral integrins resemble integrins of the baso-lateral epithelial cell surfaces. Thus, targeting proteins of the AJC may constitute a strategy that would allow viruses to bypass the physical barrier that blocks their access to integrin protein receptors expressed on the baso-lateral surface of epithelial cells.

In our patient, a final diagnosis of KVE following infection with type 1 herpes simplex virus was made. KVE has also been reported after HSV-2, coxsackievirus A16, and vaccinia virus infecting a pre-existing dermatosis. Most commonly, it presents following disseminated HSV infection in patients with atopic dermatitis. To date, the pathophysiology of KVE remains unclear. Proposed mechanisms involve both cell-mediated and humoral defects in persons with atopic dermatitis that could account for their susceptibility. In summary, we suggest that physicians should not depend solely on clinical and H&E viral cytopathic effect criteria in establishing the diagnosis of HSV infection, but should also consider immunohistochemistry for HSV 1 and 2 in the evaluation of patients with any potential KVE.

References

1. Tran T, Muelenhoff M, Saeed S, Morgan MB. The miscellaneous blistering disorders. Semin Cutan Med Surg 2003; 23:19-28.
2. Gonzalez-Mariscal L, Garay E, Lechuga S. Virus interaction with the apical junctional complex. Front Biosci 2009; 14:731-768.
3. Galen B, Cheshenko N, Tuyama A, et al. Access to nectin favors herpes simplex virus infection at the apical surface of polarized human epithelial cells. J Virol 2006; 80:12209-12218.
4. Fine, Jo-David. Management of acquired bullous skin diseases. N Engl J Med 1995; 333:1475-1484.
5. Cotell S, Robinson N, Chan L. Autoimmune blistering skin diseases. Am J Emerg Med 1999; 18:288-299.
6. Abrèu-Velez AM, Beutner EH, Montoya F, et al. Analyses of autoantigens in a new form of endemic pemphigus foliaceus in Colombia. J Am Acad Dermatol 2003; 49:609-614.
7. Abréu-Vélez AM, Yepes MM, Patiño PJ, et al. A sensitive and restricted enzyme-linked immunosorbent assay for detecting a heterogeneous antibody population in serum from people suffering from a new variant of endemic pemphigus. Arch Dermatol Res 2004; 295:434-441.
8. Atherton DJ, Marshall WC. Eczema herpeticum. Practitioner 1982; 226:971-973.
9. Bork K, Brauninger W. Increasing evidence of eczema herpeticum: analysis of seventy-five cases. J Am Acad Dermatol 1988; 19:1024-1029.
10. Anthony V. Nicola AV, Hou J, et al. Herpes simplex virus type 1 enters human epidermal keratinocytes, but not neurons, via a pH-dependent endocytic pathway. J Virol 2005; 79:7609-7616.
11. Roizman, B., and D. M. Knipe. 2001. Herpes simplex viruses and their replication, p. 2399-2459. *In* D. M. Knipe and P. M. Howley (ed.), Field's Virology, vol. 2. Lippincott Williams & Wilkins, Philadelphia, Pa.
12. Para MF, Baucke RB, Spear PG. Immunoglobulin G (Fc)-binding receptors on virions of herpes simplex virus type 1 and transfer of these receptors to the cell surface by infection. J Virol 1980; 34:512-520.
13. Lopez S, Arias CF. Multistep entry of rotavirus into cells: a Versaillesque dance. Trends Microbiol 2004; 12: 271-278.