Detection and Comparison of Viral Antigens in Measles and Rubella Rashes

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Measles and rubella skin lesions were immunocytochemically compared by the avidin-biotin-peroxidase complex method for detecting viral antigens. Cryostat sections of biopsied specimens of the skin were stained with mouse monoclonal antibodies to P protein of measles virus and to E1 protein of rubella virus. The measles virus antigen was concentrated in the corneal layer and the keratinocytes of the epidermis and in the surface part of the dermis in the biopsy secimens taken within 6 days after the onset of rash. On the other hand, the rubella virus antigen was dispersed in all parts of the dermis and the subcutaneous layer but not in the epidermis in the biopsy specimens taken within 2 days after the onset of rash. The differences in the distribution and density of the viral antigen and in the times of its detection suggest distinct patterns of spread of infection with each virus in the skin.

Measles and rubella are characterized by typical rashes, but little virological study on the pathogenesis of skin lesions in either disease has been done. In particular, few reports have dealt with the relationship between rubella virus and the skin lesion on the basis of the presence of infectious virus [1, 2] or a viral genome [3] in the rash. Several conflicting observations regarding measles rash have been reported, which suggests that the rash is caused by viral replication in the skin [4, 5] and that the rash is a manifestation of the Arthus reaction induced by the deposition of the viral antigen in the endothelium of dermal capillaries [6].

In the present study, we immunohistochemically examined the localization of measles virus (MV) and rubella virus (RV) antigens in the skin lesions to explain the possible relationship between the rash and viral synthesis in particular sites of the lesions.

Patients and Methods

Four patients with measles and four patients with rubella diagnosed at the Department of Dermatology, Showa University Hospital (Tokyo), in 1992 were enrolled in the present study. The features of the study were explained to each patient, and after obtaining consent from the patients, the following specimens were collected: punch-biopsy rash specimens taken from patients with measles up to 6 days after the onset of rash and from patients with rubella up to 2 days after the onset of rash and serum samples taken during the acute and convalescent phases of the illness. The biopsy specimens were obtained for 1 or 2 days after the period during which each virus was detected [2, 5]. The serological data for the eight patients are summarized in table 1.

Cryostat sections of the specimens stored at −20°C were fixed in anhydrous acetone for 10 minutes at −20°C. The fixed sections were stained with hematoxylin-eosin for histologic observations or were stained by the avidin-biotin peroxidase complex (ABC) method with use of mouse monoclonal antibodies (primary antibodies) to the P protein of MV, which is antigenically the most stable among five structural proteins (NP, P, M, H, and F) [7], and to the E1 protein of RV [8] for immunocytochemical observation. To find the optimal dilution of each primary antibody, Vero cells infected with the Toyoshima strain of MV and rabbit kidney (RK-13) cells infected with the M-33 strain of RV were used after fixation. The fixed tissue sections and infected cells were treated sequentially with 0.5% H_2O_2 in PBS for 30 minutes, 3% bovine serum albumin in PBS for 20 minutes, mouse monoclonal antibodies to MV P protein or RV E1 protein (1:1,000 dilution) for 60 minutes, biotinylated goat antibody to mouse IgG (1:200 dilution; Vector Laboratories, Burlingame, CA) for 60 minutes, ABC (Vector Laboratories) for 30 minutes, and 0.5 mg of 3, 3′-diaminobenzidinetetrahydrochloride ($C_{12}H_{14}N_4 \cdot 4HCl$; WAKO Pure Chemical Industries, Japan)/mL in 0.02% H_2O_2 as the chromogen. After staining, the sections were counterstained with hematoxylin. Skin specimens from normal humans and from autopsy patients infected with parvovirus served as negative controls.

Results

The histologic and immunocytochemical features observed in measles and rubella skin lesions markedly differed from each other. In measles skin lesions, severe histologic changes were observed, including spongiosis and necrosis with mononuclear cell infiltration in the keratinocytes localized in the epidermis; however, there was only slight mononuclear cell infiltration in the dermis and the subcutaneous layer. No multi-

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Table 1. Summary of data for eight patients with measles or rubella.

Disease, case no., age (y)/sex	Previous infection	Vaccination	Time of skin biopsy (d after the onset of rash)	CF/HI/NT titer	
				Acute phase	Convalescent phase
Measles					
1, 8/M	No	Not known	2	<4/16/16	64/128/64
2, 16/M	No	No	3	<4/16/32	32/128/48
3, 16/F	No	No	5	<4/<8/16	16/64/24
4, 16/F	No	No	6	32/8/<4	128/256/64
Rubella					
1, 34/F	Yes	No	1	<4/<8/ND	<4/512/ND
2, 37/M	Yes	No	1	<4/<8/ND	<4/256/ND
3, 37/F	Yes	No	1	<4/<8/ND	<4/512/ND
4, 38/M	Not known	No	2	<4/<8/ND	<16/256/ND

NOTE. CF/HI/NT = complement-fixing antibody/hemagglutination-inhibiting antibody/neutralizing antibody; ND = not done.

nuclear giant cells were found in any skin specimen. The MV antigen was localized in the corneal layer and in spongiotic keratinocytes of the epidermis, and compared with viral antigen in the other lesions, the viral antigen was dense in the surface part of the dermis, especially in the papillary layer (figure 1). The distribution pattern and relative amount of the MV antigen were similar in all cases in which the skin lesions were biopsied 2, 3, 5, and 6 days after the onset of rash.

In contrast, in rubella skin lesions, mononuclear cell infiltration was generally observed only in the dermis. The RV antigen was detected along with mononuclear cell infiltration extending from the dermis to the subcutaneous layer, and the RV antigen tended to be distributed in the layer of the dermis deeper than where the MV antigen was detected (figure 2). The RV antigen was generally less dense than the MV antigen in any part of the skin lesion. The RV antigen was detected in all four cases in which the specimen was taken within 2 days after the onset of rash.

The histologic and immunocytochemical findings for measles and rubella skin lesions are summarized in table 2. Negative controls consisting of skin specimens from normal humans and from autopsy patients infected with parvovirus showed a lack of specific staining. The specimens taken from measles rashes and treated with antibody to RV and those taken from rubella rashes and treated with antibody to MV were not stained.

Discussion

We demonstrated the presence of MV and RV antigens in measles and rubella skin lesions, respectively, with the ABC

Table 2. Comparison of rashes caused by measles and rubella.

Layer of skin, finding	Measles rash	Rubella rash
Epidermis		
Histopathologic change	Spongiosis and necrosis in the keratinocytes; severe inflammation with mononuclear cell infiltration	No particular change
Viral antigen	Positive in the corneal layer, the keratinocytes, and the hair follicles	Negative
Dermis, papillary layer		
Histopathologic change	Mononuclear cell infiltration	Mononuclear cell infiltration
Viral antigen	Positive	Positive, less than the measles virus antigen
Dermis, reticular layer		
Histopathologic change	Mononuclear cell infiltration	Mononuclear cell infiltration
Viral antigen	Negative	Positive
Subcutaneous layer		
Histopathologic change	Negative	Mononuclear cell infiltration
Viral antigen	Negative	Positive

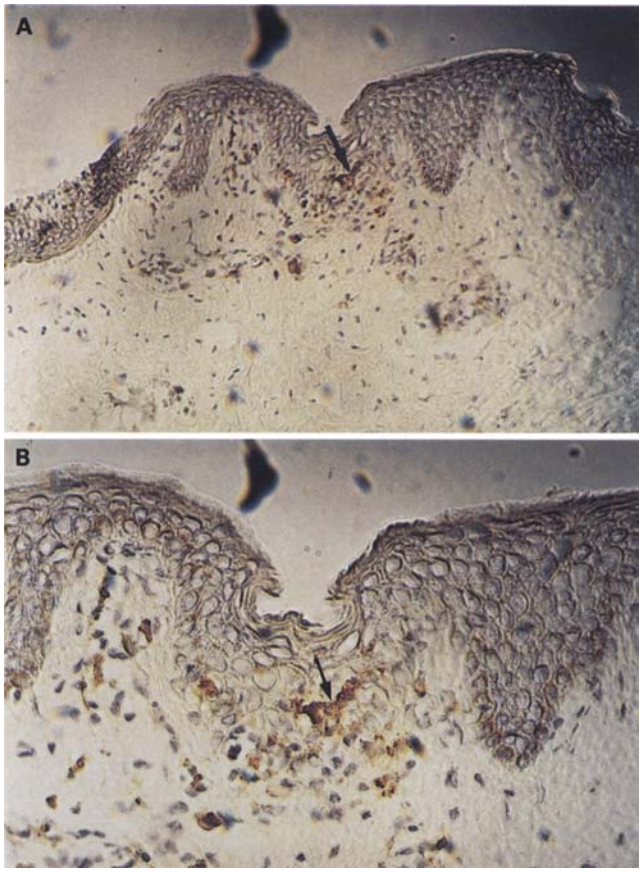

Figure 1. A skin lesion biopsied 2 days after the onset of measles rash. This skin section was stained by the avidin-biotin-peroxidase complex method with use of mouse monoclonal antibody to P protein of measles virus and was counterstained with hematoxylin. Spongiosis and necrosis with mononuclear cell infiltration in the keratinocytes are observed, with dense viral antigen localized in the keratinocytes and the papillary layer of the dermis. *A*: magnification, × 156; *B*: magnification, × 312. Arrows show the same cells.

method. Olding-Stenkvist and Bjorvatn [5] reported that measles rash was directly caused by local MV replication in the skin; they showed the presence of the viral antigen in the surface epithelium, skin appendages, and the dermis in biopsied specimens taken within 4 days after the onset of rash. The skin specimens taken 5–6 days after the onset of rash were positive for viral antigen in our study but negative in their investigation. Because the MV antigen was detectable in the skin lesions taken during late stages of the rash in the present study, our method may be more sensitive than the method used by Olding-Stenkvist and Bjorvatn. Our findings may also be supported by the hypothesis of Suringa et al. [4] that measles rash is directly caused by infection with MV as evidenced by the presence of microtubular viral aggregates in the epithelium. These investigators observed syncytial giant cells or typical inclusions; however, we did not observe these cells. It was also reported that the MV antigen was detected in the granular layer of the epidermis in autopsy specimens from patients who died of acute measles [9]. On the basis of these results and our findings, it appears that the major target of MV is the epithelium.

Kimura et al. [6] reported that measles rash might be the result of a primary antigen-antibody reaction in the endothelium of dermal capillaries, because neither typical inclusions nor viral nucleocapsids were detected in the epidermis. However, these findings disagree with the above-mentioned results, including our own. We conclude that measles rash is due to viral replication in such a localized cell layer of the skin as the corneal layer, in keratinocytes of the epidermis, and in the papillary layer of the dermis.

In previous reports, it was assumed that rubella rash was associated with viral infection on the basis of the presence of infectious virus [1, 2] and a viral genome [3] in the skin lesion. These reports suggested the presence of the virus in the whole skin tissue, but to our knowledge no viral antigen has been detected in situ in skin lesions. The present study demonstrated

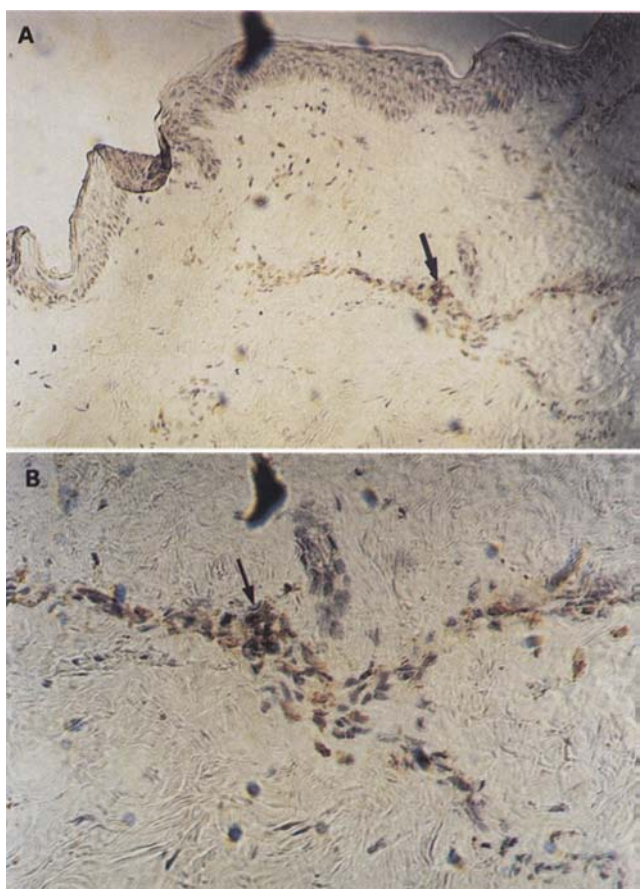

Figure 2. A skin lesion biopsied 1 day after the onset of rubella rash. This skin section was stained by the avidin-biotin-peroxidase complex method with use of mouse monoclonal antibody to E1 protein of rubella virus. Rubella virus antigen is localized to lines of infiltrated mononuclear cells along vessels only in the dermis. *A*: magnification, × 156; *B*: magnification, × 312. Arrows show the same cells.

that the RV antigen was widely distributed from the surface part of the dermis to the subcutaneous layer, being more abundant in the deeper part of the dermis and the subcutaneous layer; however, no viral antigen was detected in the epidermis. Thus, the mode of distribution of the RV antigen was in contrast with that of the MV antigen concentrated in the surface part of the dermis. On the basis of such a difference, the targets of the two viruses may be different, thereby resulting in different patterns of viral spread.

There may be two possible mechanisms by which viral infection causes rash: by the virus directly and by some immunologic factor such as an interaction between the virus and the host. On the basis of our findings of spongiotic keratinocytes and mononuclear cell infiltration as well as the detection of the MV antigen in the epidermis, we hypothesize that the infiltrated lymphocytes attacked the infected keratinocytes, which caused spongiosis. Therefore, measles rash may be directly caused by viral replication and by the immune response of lymphocytes to the affected keratinocytes of the epidermis. We assume that rubella rash is caused by a mechanism similar to that for measles rash since the RV antigen was accompanied by mononuclear cell infiltration in the dermis and the subcutaneous layer.

We conclude from this study that the presence of the viral antigen in measles and rubella rashes indicates direct viral infection of the skin and that the distribution of each viral antigen is tissue-specific.

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