An Immunofluorescent and Electron Microscopic Study of Measles Skin Eruptions

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KIMURA, A., TOSAKA, K. and NAKAO, T. An Immunofluorescent and Electron Microscopic Study of Measles Skin Eruptions. Tohoku J. exp. Med., 1975, 117 (3), 245-256 — Immunofluorescent study was attempted to determine whether or not virus antigen were present in the epidermis of measles eruptions. The electron microscopic observations of the same materials were also performed to detect viral localization in affected skins. The failure to detect any virus antigen in affected epidermis throughout all eruptive stages seems to be sufficient evidence to conclude that measles rash is not a manifestation of viral replication in the epidermis. Dotted fluorescences were detected in a specimen taken at pre-eruptive day in capillary endothelium of dermis. At the same stage, microtubular structures which were probably identical with measles virus nucleocapsids occurred in capillary endothelium under the electron microscopic observations. It is concluded that measles rash is possibly caused by an antigen-antibody reaction of Arthus type. On very rare occasions, measles virus nucleocapsids were found in the cytoplasm of dermal fibroblast in the vicinity of dermal capillary. Ultrastructural features of these nucleocapsids were demonstrated to be identical to features of microtubular structures found in endothelial cells.

The interpretation that measles eruptions are a manifestation of an Arthus reaction elicited by viral antigen in the endothelial cells of dermal capillaries has already been reported elsewhere (Kimura et al. 1975). This interpretation was supported by the following reasons; 1) neither typical inclusion nor viral nucleocapsids could be detected in any part of the epidermis throughout all eruptive stages, 2) measles virus-like microtubular structures were frequently observed in capillary endothelium at the pre- and early-eruptive stages, and 3) there was no obvious virus infection in dermal portion which apparently took place in cases of varicella skin lesions prior to epidermal virus infection (Kimura 1972).*

The present study was performed to examine the localization of virus antigen in skin lesions by immunofluorescent technique. The possible identity of microtubular structures and measles virus nucleocapsids was also discussed.

MATERIALS AND METHODS

1) Biopsied specimen: Skin biopsies were made on thirteen measles patients at intervals from pre-eruptive day till 7th eruptive day. Details of biopsied specimens are

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* Presented before the 24th Meeting of Pediatrician of North-Japan, Niigata, September 23rd, 1972.
TABLE 1. Data of biopsied materials

<table>
<thead>
<tr>
<th>Case</th>
<th>Age (year)</th>
<th>Sex*</th>
<th>Day of skin biopsy (after the onset of rash)</th>
<th>C.F. titer† before</th>
<th>C.F. titer† after</th>
<th>Site of biopsy‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5</td>
<td>M</td>
<td>pre-eruptive day†§</td>
<td>&lt;1:4</td>
<td>1: 256</td>
<td>D.L.</td>
</tr>
<tr>
<td>2</td>
<td>3</td>
<td>F</td>
<td></td>
<td>&lt;1:4</td>
<td>1: 64</td>
<td>D.L.</td>
</tr>
<tr>
<td>3</td>
<td>3</td>
<td>M</td>
<td></td>
<td>1:4</td>
<td>1:1024</td>
<td>D.L.</td>
</tr>
<tr>
<td>4</td>
<td>6</td>
<td>M</td>
<td>1st eruptive day</td>
<td>1:4</td>
<td>1: 128</td>
<td>D.L.</td>
</tr>
<tr>
<td>5</td>
<td>3</td>
<td>M</td>
<td></td>
<td>&lt;1:4</td>
<td>1: 512</td>
<td>B</td>
</tr>
<tr>
<td>6</td>
<td>1</td>
<td>F</td>
<td>2nd eruptive day</td>
<td>&lt;1:4</td>
<td>1: 128</td>
<td>D.L.</td>
</tr>
<tr>
<td>7</td>
<td>2</td>
<td>M</td>
<td></td>
<td>&lt;1:4</td>
<td>1: 256</td>
<td>D.L.</td>
</tr>
<tr>
<td>8</td>
<td>2</td>
<td>M</td>
<td>3rd eruptive day</td>
<td>&lt;1:4</td>
<td>1: 256</td>
<td>B</td>
</tr>
<tr>
<td>9</td>
<td>1</td>
<td>M</td>
<td></td>
<td></td>
<td></td>
<td>B</td>
</tr>
<tr>
<td>10</td>
<td>1</td>
<td>F</td>
<td></td>
<td></td>
<td></td>
<td>D.L.</td>
</tr>
<tr>
<td>11</td>
<td>5</td>
<td>M</td>
<td>4th eruptive day</td>
<td>1:4</td>
<td>1: 128</td>
<td>D.L.</td>
</tr>
<tr>
<td>12</td>
<td>2</td>
<td>F</td>
<td>5th eruptive day</td>
<td></td>
<td></td>
<td>D.L.</td>
</tr>
<tr>
<td>13</td>
<td>1</td>
<td>M</td>
<td>7th eruptive day</td>
<td>&lt;1:4</td>
<td>1: 256</td>
<td>B</td>
</tr>
</tbody>
</table>

* M, male; F, female.
† Paired sera were taken at intervals ranging from 6 days to 14 days.
‡ D.L, dorsolateral region; B, buttocks.
§ Koplik's spots (+), in some patients, rash already appeared on their face and neck.

summarized in Table 1.

2) Electron microscopy: Small pieces of each specimen were treated using the same methods as previously reported (Kimura et al. 1975) and then examined in a JEM-100 C electron microscope (JEOL Co. Ltd).

3) Antisera and fluorescein-labeling: Five ml of antimeasles-virus rabbit sera (Chiba Serum Institute) were used in preparation of the study. Antisera were precipitated with half their volume of saturated ammonium sulfate solution and crude globulin fraction was labeled with fluorescein isothiocyanate (FITC; Dickinson Co.) according to the method of Kawamura (1966).

4) Immunofluorescence: Remnant biopsied specimens were stocked at -70°C till the experiment was carried out. Every specimen was examined within a week after its picking. Frozen sections were cut at 4 μ with a cryostat and fixed in cold acetone for 30 min. The sections were stained directly with fluorescent reagin at 37°C for one hr in a moist chamber. The stained sections were examined in a FM-200 A microscope (Tiyoada Kogaku Co.) equipped with HBO 200 light source and a dark-ground condenser. Photographs were taken on Neopan SSS (Fuji Photographic Co.) 150 day light film. The fluorescent reagin had been pre-tested with a study using infected cultured cells with measles virus (Tosaka and Kimura 1971).

5) Control: Control, non-eruptive skins were taken from two patients; 3-year-old girl with hypothyroidism and 1-year-old boy with infantile myoclonic seizures. These specimens were tested with the same fluorescent reagin.

RESULTS

Immunofluorescent study. There was no detectable specific fluorescence in epidermal portion throughout all eruptive stages (Figs. 1–3). Even at the maximum eruptive stage (3rd or 4th eruptive day), specific fluorescence could not
be detected in any part of the epidermis (Figs. 2 and 3).

In one section taken at pre-eruptive day, dotted fluorescence was detected in the dermal portion which seemed to correspond to endothelial cells of dermal capillary (Fig. 4). This tiny fluorescence was also found in another specimen taken at the first eruptive day in the cytoplasmic portions of mononuclear cell in the dermis (Fig. 5).

**Electron microscopy.** Microtubular structures which were already fully mentioned by Kimura et al. (1975) were frequently observed in the specimen taken at pre- and first-eruptive days (Figs. 6 and 7). These microtubuli showed the tendency to distribute with close relation to endoplasmic reticulum (ER) or mitochondria (Figs. 6 and 7). They were, however, often found freely distributed in the cytoplasm.

In very rare instances, measles virus seemed to be replicated in dermal fibroblasts in the vicinity of dermal capillary. In one specimen of the first eruptive day, virus nucleocapsids were found in the cytoplasmic portions of a dermal fibroblast (Fig. 8). There was no evidence of giant cell formation. At the peripheral portions of their assembly, these nucleocapsids showed a close relation to ER and even to mitochondria (Figs. 8 and 9). The morphological features and the size of these nucleocapsids under the higher magnification were very similar to those of intraendothelial microtubuli (Figs. 7 and 9).

Materials which seemed to be composed of microtubular structures were observed in the cytoplasmic vacuoles of dermal macrophages in some ultra-thin sections of the first and second eruptive days (Fig. 10).

**DISCUSSION**

An interpretation that measles eruptions are possibly raised by antigen-antibody reaction of Arthus type elicited by viral antigen in capillary endothelium has already been discussed in detail (Kimura et al. 1975). In the present study, viral antigen was detected by immunofluorescent technique in capillary endothelium in a specimen taken at pre-eruptive day. The possible identity between measles virus and intraendothelial microtubuli is strongly supported by this finding. No viral antigen, on the other hand, could be detected in any portion of the epidermis throughout all eruptive stages; the active virus synthesis in epidermal cells causing eruptions as reported by Suringa et al. (1970) seems unlikely to take place in measles skin lesions.

Although virus nucleocapsids were found in dermal fibroblasts (Fig. 8), the occurrence of this kind of infected cell was extremely rare. There was, in fact, only one cell bearing virus nucleocapsids among a large number of ultra-thin sections which were examined. The rarity of the occurrence of these infected cells in the dermis is supported by the finding of immunofluorescent study. It was only an exceptional occurrence that virus antigen was detected in the cytoplasm of dermal fibroblasts (Fig. 5) despite careful examination of many sections. There was no
evidence of giant cell formation nor the occurrence of typical inclusion in any part of the dermis or epidermis throughout all eruptive stages (Kimura et al. 1975).

In contrast to measles eruptions, in varicella skin lesions in which active virus replication took place in epidermal cells resulting in vesicle formation (Kimura et al. 1972), a considerable proportion of dermal fibroblasts were infected by the virus at an early stage of eruptions (Kimura 1972).* Virus infection would take place actively in some dermal portions prior to viral invasion of the epidermis, but this kind of dermal virus infection would not occur in measles skin lesions.

Regarding this, it excites special interest that materials which seemed to be composed of microtubular structures were found in the cytoplasmic vacuoles of dermal macrophages (Fig. 10). No specific virus antigen was detected in any dermal macrophage by immunofluorescent technique; however, these cytoplasmic portions would be too small to be detected by light microscopic observations. We regard these materials as phagocytized measles virus nucleocapsids.

On the basis of the above-mentioned findings, the principal pathological process which mediates the appearance of the rash would be the vascular damage by antigen-antibody reaction as already reported (Kimura et al. 1975). Viral replication seemed to take place in dermal fibroblasts only in exceptional instances and virus released into the dermis from infected cells would be phagocytized rapidly by dermal macrophages. In addition to these phagocytoses by macrophages, raising humoral antibody would also inhibit the extension of infection; no virus could infect any part of the epidermis.

The morphological features and the size of intra-endothelial microtubuli and viral nucleocapsids are well corresponding to each other (Figs. 7 and 9). These are tubular in appearance and about 18–20 nm in width. They showed the same tendency to relate closely with ER and mitochondria at the peripheral portions of their assembly.

We conclude that the intra-endothelial microtubuli are probably identical with measles virus nucleocapsids and these intra-endothelial viruses would play a principal role in the appearance of the rash.

Acknowledgment

We would like to thank Prof. T. Onoe of Department of Pathology of Sapporo Medical College for his kind and helpful advice.

References

Fig. 1. A photograph of immunofluorescent examination made on skin lesions of 1st eruptive day. No specific fluorescence is detectable. d, dermis; e, epidermis. × 300.

Fig. 2. Immunofluorescent study on 3rd eruptive day. Virus antigen is not visible. h, hypertrophied horny layer. × 300.

Fig. 3. Another section of 3rd eruptive day. Specific fluorescence is not visible. × 1,000.

Fig. 4. Dermal portions of a specimen taken at pre-eruptive day. At the center of this photograph, non-specific fluorescences are observed on clustered red blood cells (black arrow). Dotted specific fluorescences are seen in endothelial cells surrounding these red blood cells (white arrows). × 1,500.
Fig. 5. A dermal portion of a specimen taken on 1st eruptive day. Fluorescences are visible in the cytoplasmic portions of mononuclear cell (arrow). $\times 1,500$.

Fig. 6. A photograph of electron microscopic study of a specimen of pre-eruptive day. Microtubular structures are observed in the cytoplasmic portions of capillary endothelial cell (arrow). N, nucleus. $\times 40,000$.

Fig. 7. Microtubular structures are observed in the cytoplasmic portions in the vicinity of a mitochondrion. First eruptive day. Bm, basement membrane; Mt, mitochondrion; N, nucleus. $\times 45,000$. 
Fig. 8. This shows measles virus assembly observed in the cytoplasm of a dermal fibroblast. First eruptive day. En, capillary endothelial cell; Fb, fibroblast. × 24,000.

Fig. 9. Higher magnification view of virus nucleocapsids. They are closely related to ER (arrows) and mitochondria at the peripheral portions of their assembly. × 45,000.
Fig. 10. Materials which seem to be composed of microtubular structures are observed in the cytoplasmic vacuole (arrow) of a dermal macrophage. × 30,000.