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What is This?
Pathology of Measles in Rhesus Monkeys

W. C. Hall, R. M. Kovatch, P. H. Herman, and J. G. Fox

Medical Sciences Laboratories, Aerobiology and Evaluation Laboratories, Department of the Army, Fort Detrick, Frederick, Md.

Abstract. Skin biopsies were taken from 16 monkeys with measles rash. Histopathological changes consisted of multinucleated epithelial giant cells in the epidermis and hair follicles, proliferative and degenerative changes of the epidermis, and mild inflammation of the dermis. Necropsies were performed on two monkeys with a typical rash and on 26 additional monkeys that died during outbreaks in the colony. Lesions attributable to rubeola virus were found in the respiratory system, lymphoid system, gastrointestinal tract, salivary glands, thyroid gland, liver, pancreas, and urinary system. In these organs the finding that was characteristic of measles was the presence of syncytial giant cells, some of which contained both intranuclear and cytoplasmic inclusions. The significance of syncytia and the mechanism of their formation are discussed.

Measles in monkeys presents various clinical syndromes. Often, no sign is evident and the only indication of infection is a positive serological titer [26]. In experimental studies some monkeys had the entire clinical syndrome of fever, leucopenia, conjunctivitis, Koplik's spots on labial and buccal mucous membranes, and finally a red maculopapular rash that covered most of the anterior body surface [3].

Meyer et al. [17] studied the disease in Indian Rhesus monkeys and concluded that the animals are free of measles in their natural habitat, but a colony becomes infected after exposure to a primate with the disease. This hypothesis was supported by Shishido [21], who showed that very few monkeys imported from southeastern Asia had titers to rubeola on arrival into his colony, but often within a few weeks every one had humoral antibodies to the disease.

Lesions vary with the stage of the disease in both man and monkeys. Multinucleated giant cells with and without inclusions have been described in the
respiratory tract [12, 19], lymph nodes [19], Peyer's patches [7], tonsils [19], urinary bladder [20], brain [1], and skin [23]. Other lesions attributable to the virus have also been described in the heart [4], eye [9], and oral mucous membranes [10]. Except for the lymph nodes and lungs, lesions in other viscera and the skin of monkeys have been infrequently or only poorly described.

This paper describes pathological changes observed at necropsy and histopathological changes in skin biopsied when the rash was present in spontaneous cases of measles in a colony of Rhesus monkeys imported from India.

**Materials and Methods**

Rhesus monkeys were imported directly from India to the Fort Detrick Animal Farm. During a 2-year period several outbreaks of measles were recognized clinically. During two of these outbreaks 18 monkeys with rash were selected for morphological studies; two were killed and necropsied, and skin biopsies were taken from the remaining 16. Five of these 16 monkeys had been previously immunized with rubeola vaccine on the date of arrival into the colony. These were included in this study because rashes and other clinical signs were observed simultaneously in vaccinates and controls within 7 days of arrival, an interval less than the reported incubation period for measles. Monkeys selected for biopsy were placed in a cataleptoid state with phencyclidine, 1 mg/kg of body weight, and a skin lesion was removed by elliptical incision. The measles outbreaks were substantiated by demonstrating a rise in serum titer to rubeola virus. Paired blood samples were taken from 13 of the biopsied monkeys. The first sample was withdrawn at the time of biopsy and another, 3 to 4 weeks later. Serum was separated, and hemagglutination inhibition (HI) titers were determined. Samples were not taken from the two necropsied monkeys and two of the biopsied monkeys. One other biopsied monkey died of typhlitis before paired serum samples could be obtained.

Tissue sections were reviewed retrospectively from monkeys that died during previous outbreaks and were coded as having giant-cell pneumonia or measles. A diagnosis of measles was made in 26 monkeys in which there were characteristic pulmonary or lymphoid changes. All tissues were fixed in 10% formalin, embedded in paraffin, and processed in the usual manner. In addition to hematoxylin and eosin stains, skin sections were also stained by the Gomori methenamine silver method to identify fungi [16]. Selected sections of lymph nodes were stained for acid-fast organisms [16].

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1 Measles virus vaccine, live, attenuated (Schwarz strain) lyophilized chick embryo tissue culture origin. Dow Chemical Co., Indianapolis, Ind.

2 Dr. S. S. Kalter, Southwest Foundation for Research and Education, San Antonio, Tex.
Results

Clinical Findings in Biopsied Monkeys

Eighteen monkeys with a red maculopapular rash were selected for skin biopsy. All monkeys had facial erythema, and 16 of the monkeys had conjunctivitis. The rash was distributed over the chin, anterior parts of the thorax and abdomen, and the anteromedial surfaces of the thighs and groin. The focal skin lesions were usually 0.25 to 0.50 cm apart but in some areas were confluent. The oral cavities of seven monkeys were examined within 48 h of the onset of the rash. Six of the seven had raised white foci, 1 to 2 mm in diameter, on the gingival, buccal, and labial mucosa. The lesions were interpreted as Koplik's spots [3]. Most were on the labial surfaces of the gingiva.

Serological Findings

Serological results in monkeys with rash are summarized in table I. Seven of eight unvaccinated monkeys had a fourfold increase in HI titer to rubeola in a 3- to 4-week interval. One monkey that had the initial serum sample and biopsy taken 4 days after the onset of rash had paired serum HI titers of greater than 1:320. Three unvaccinated monkeys used in the morphological studies could not be evaluated because paired samples of serum were not obtained. The five vaccinated monkeys developed rash at the same time as other unvaccinated monkeys of the same group. Titers from both vaccinated and unvaccinated monkeys of this group increased from less than 1:10 to 1:160 or greater after a 4-week interval.

Table I. Hemagglutination-inhibition titers of rubeola in Rhesus monkeys with a rash

<table>
<thead>
<tr>
<th>Number of animals</th>
<th>Initial titer</th>
<th>Final titer</th>
</tr>
</thead>
<tbody>
<tr>
<td>5 (2)</td>
<td>&lt;10</td>
<td>&gt;320</td>
</tr>
<tr>
<td>4 (3)</td>
<td>&lt;10</td>
<td>&gt;320</td>
</tr>
<tr>
<td>1</td>
<td>10</td>
<td>80</td>
</tr>
<tr>
<td>1</td>
<td>40</td>
<td>&gt;320</td>
</tr>
<tr>
<td>1</td>
<td>80</td>
<td>&gt;320</td>
</tr>
<tr>
<td>1</td>
<td>&gt;320</td>
<td>&gt;320</td>
</tr>
</tbody>
</table>

1 Dilutions expressed as reciprocal values.

* Numbers in parentheses are those vaccinated against rubeola.
Pathological Findings

Skin

Skin sections were examined from all monkeys with rash. The most specific changes were in the epidermis and hair follicles. There were various degrees of acanthosis of the epithelium. Foci of hyperkeratosis and parakeratosis were frequent. The parakeratotic changes in the epithelium were continuous with similar parakeratotic areas in the superficial parts of some of the follicles. Frequently, there was necrosis of hair follicles with only the external root sheath remaining (fig. 1). Microabscesses 100 to 200 \( \mu \text{m} \) in diameter were confined to the epidermis and follicles, and did not extend into the dermis (fig. 2). The most specific change was the presence of syncytia of epithelial cells with three to 20 nuclei in the epidermis (fig. 2, 3) and follicles. These multinucleated cells were found in 15 of the 18 monkeys. Syncytia were occasionally found in parakeratotic epithelium and in parakeratotic areas in the follicles (fig. 3, 4). No inclusions were found.

Dermal changes were nonspecific. There were vascular congestion, edema of the corium, and perivascular cuffing by mononuclear cells. Neutrophils and eosinophils were occasionally found in the cuffs. The perivascular reaction rarely extended into the epidermis.

Respiratory System

Lung

All pulmonary sections were obtained from monkeys at necropsy. Hemagglutination inhibition titers were not determined for any of these animals. Two monkeys had the characteristic skin lesions of measles. These monkeys and 19 of the 26 remaining monkeys had giant-cell pneumonia.

In mild cases of giant-cell pneumonia, there was peribronchiolar localization of lesions (fig. 5). Diffuse involvement of pulmonary interstitium occurred in severe cases. There was a minimal serous exudate in alveoli and excess mucus in bronchioles. Very little cellular exudate was noted, but, when

\[ \text{Fig. 1. Skin. Acute folliculitis, perivascular cuffing by mononuclear cells and edema of the corium. Neutrophils have migrated from the dermis into the epidermis. HE, } \times 109. \]

\[ \text{Fig. 2. Skin. Syncytial epithelial giant cell and micro-abscess in the epidermis. HE, } \times 350. \]

\[ \text{Fig. 3. Skin. Acanthosis and parakeratosis of the epidermis; inflammation of the corium. Epithelial syncytia are present in the parakeratotic and Malpighian layers of the epidermis. HE, } \times 109. \]
Fig. 4. Hair follicle. Parakeratosis of the follicle with an epithelial syncytium in the parakeratotic layer. HE, × 350.

Fig. 5. Giant-cell pneumonia with peribronchiolar giant cells (arrow) and inflammatory exudate. HE, × 35.

Fig. 6. Intra-alveolar syncytial giant cells with cytoplasmic and intranuclear inclusions. HE, × 350.
present, the majority of the cells were mononuclear, except in bronchioles where neutrophils were found. The most striking changes in the lung were hypertrophy and hyperplasia of alveolar epithelial cells, with syncytial formation.

Most syncytia had intranuclear and cytoplasmic inclusions (fig. 6). The intranuclear inclusions were single, homogenous, and eosinophilic. Some occupied the entire nucleus, but most had a clear halo surrounding the inclusion. Margination of chromatin was evident in all affected nuclei. The multiple cytoplasmic inclusions were of variable size, stained magenta with HE, and were irregularly shaped. Most were surrounded by a clear halo. They were present in most giant cells. Both intranuclear and cytoplasmic inclusions could also be found in single epithelial cells.

Secondary bacterial invasion complicated many cases of pneumonia, and caused necrosis and purulent inflammation.

Trachea, Bronchi, Bronchioles

All monkeys with giant-cell pneumonia had focal metaplasia of the tracheal and bronchial epithelium to nonciliated, low columnar, or flattened epithelium. Eight monkeys with giant-cell pneumonia and an additional six without it had syncytia of tracheal, bronchial, or bronchiolar epithelium (fig. 7). Inclusions similar to those in the lung were seen in these syncytia in half the monkeys.

Lymphoid System

Lymphoid tissues of 27 monkeys were sectioned, and changes characteristic of measles were found in lymphoid organs of 13. The different types of giant cells described by Nitsch et al. [19] were observed when the lymphoid tissues of these monkeys were reviewed: (1) lymphoid giant cells (Warthin-Finkeldey cells) (fig. 8); (2) reticular giant cells; (3) plasmacytic giant cells; and (4) phagocytic giant cells (fig. 9). Both eosinophilic intranuclear and cytoplasmic inclusions were found in the reticular giant cells and rarely in phagocytic giant cells. Occasionally, a syncytial giant cell that was composed of both mature lymphocytes and reticular cells in a single syncytium was found.

A few syncytial giant cells were found in lymphoid tissue of the lamina propria of the stomach and in lymphoid nodules of the small and large intestines. In addition to the syncytial cells, the only other change in lymphoid tissues was focal depletion of lymphocytes, usually in the areas of reticular giant-cell formation.
Fig. 7. Syncytial giant cells of the lining epithelium of a bronchiole. A mild inflammatory exudate is present in the ducts. HE, ×109.

Fig. 8. Lymphoid giant cell (Warthin-Finkeldey cell) in a lymph node. HE, ×550.

Fig. 9. Phagocytic giant cells. Nuclei of the syncytia resemble reticular cells. HE, ×350.

Fig. 10. Glossitis with epithelial syncytia in parakeratotic part. HE, ×109.

Fig. 11. Focal necrosis and loss of the epithelium of the large intestine. Syncytial cells (arrows) are difficult to discern at this magnification. HE, ×109.

Fig. 12. Salivary gland with a syncytial giant cell of parenchymal epithelium. Both intranuclear and cytoplasmic inclusions are present in the syncytium.
Gastrointestinal System

Oral Cavity and Esophagus

Koplik's spots were not examined histologically but six of nine tongues and six of 14 esophagi had changes similar to those described for the skin. There was acanthosis, parakeratosis, and hyperkeratosis of the epithelium. Epithelial syncytia were occasionally found in superficial layers of the mucosa (fig. 10). The lamina propria had mild inflammatory changes characterized by mononuclear infiltrations similar to those in the skin.

Intestines

The intestinal tract was examined histologically in 21 monkeys. Indistinct syncytial giant cells formed of intestinal epithelium were found in the crypts of the colon in one monkey and of small intestines in two. The cytoplasm of the syncytia was slightly eosinophilic, and nuclei were basophilic and homogenous. No inclusion was found. There was mild focal necrosis of glandular epithelium with the syncytial cells in the colon (fig. 11).

Salivary Gland

Two monkeys with accompanying giant-cell pneumonia had syncytia of the salivary parenchymal epithelium which contained both intranuclear and cytoplasmic inclusions (fig. 12) similar to those in the lung. Focal infiltrations of lymphocytes were present around some of the syncytia of one monkey. No lesion was observed in the salivary glands of eight other monkeys.

Urinary System

Syncytial epithelial giant cells were found in the epithelium of the urinary bladder (fig. 13) and/or renal pelvis in eight of 26 monkeys. Cytoplasmic inclusions were sometimes seen in the syncytia, and the nuclei were basophilic and homogenous.

Liver

Twenty-one of 27 monkeys with pulmonary or lymphoid lesions characteristic of measles had numerous syncytial giant cells formed of hepatocytes (fig. 14). The syncytia contained up to 20 nuclei. The cytoplasm of many syncytia was more eosinophilic than that of unaffected cells. Nuclei within syncytia occasionally contained distinct eosinophilic inclusions. More frequently, however, the nuclei of the syncytia and surrounding hepatocytes contained ill-defined, homogenous, weakly basophilic masses (fig. 15). Such syncytia had a multifocal distribution. In a few cases separation of hepatocytes disrupted the continuity of the hepatic cords.
Syncytial giant cells without inclusions were found in intrahepatic biliary epithelium in five of the 27 monkeys.

**Pancreas**
Of 14 pancreases, syncytial giant cells were observed in the ductal epithelium of two and parenchymal epithelium of two others. No inclusion was detected.

**Circulatory System**

**Heart**
The heart was sectioned in 26 monkeys. Three monkeys with giant-cell pneumonia and one with lymphoid giant cells had mild interstitial myocardial...

*Fig. 13.* Epithelial syncytia in the mucosa of the urinary bladder. HE, ×350.

*Fig. 14.* Hepatocellular syncytia. HE, ×125.

*Fig. 15.* Hepatocellular syncytium with ill-defined intranuclear masses. HE, ×550.

*Fig. 16.* Syncytium of thyroid epithelium. There is margination of chromatin in most syncytial nuclei. HE, ×550.
tis characterized by small multifocal accumulations of lymphocytes in the interstitium.

**Pulmonary Arteries**

Syncytial cells formed of endothelium were found in small branches of pulmonary arteries in the lungs of two monkeys. No inclusion was detected.

**Brain**

Brains of eight monkeys were examined. Although there was no well-defined alteration, three had indistinct syncytial giant cells. In two monkeys they were found in the pia mater of the mid-brain and cerebral hemisphere and in the third monkey, in the choroid plexus of the fourth ventricle.

**Eye**

Eyes of three monkeys were examined. One had chronic conjunctivitis and acute dacryoadenitis of undetermined cause.

**Thyroid**

Syncytial giant cells of thyroid epithelium were found in three monkeys with giant-cell pneumonia (fig. 16). Both cytoplasmic and intranuclear inclusions were found in some syncytia.

**Discussion**

The fourfold increase in the HI titer, when coupled with the clinical signs and characteristic lesions, supports the diagnosis of measles in these monkeys. One monkey had an initial HI titer greater than 1:320 on the fourth day of rash. It probably contracted measles at least 2 weeks before the initial serum sample was taken. Experimentally, the rash is usually not seen before the tenth day after parenteral inoculation and is thought to occur later in spontaneous measles [12]. The five monkeys vaccinated against rubeola developed clinical signs and lesions of measles and had increases in titer similar to other monkeys of the same group. They probably were incubating the disease at the time of vaccination. Experimentally, two other viruses, rinderpest and canine distemper, also cause a rise in titer to measles [8, 26]. The titers, however, are lower than those produced by measles virus. Lesions are not found in monkeys experimentally infected with rinderpest virus, and the natural infection of monkeys by rinderpest in endemic areas is not thought to occur
Comparable information about monkeys infected with canine distemper virus is not available.

Rubeola is caused by one of the paramyxoviruses, which tend to cause the formation of syncytia both in vitro and in vivo [25]. The agent is pantropic and will infect cells derived from the three germinal layers [18]. In tissue culture, both live and killed measles viruses cause giant cells to form by a successive fusion of adjacent cells [5, 13]. This mechanism was supported in our studies by the finding of lymphoid syncytia containing nuclei of distinctly different cell types—lymphocytes and reticulum cells.

All paramyxoviruses form eosinophilic cytoplasmic inclusions, but, as far as is presently known, only in measles, distemper, rinderpest, and parainfluenza are intranuclear inclusions formed as well [25]. The inclusions caused by measles virus have been described ultrastructurally [24] and can be stained only in the early stages, by immunofluorescent means [1, 14].

Clinically, the most significant lesions of measles are those in the lung. The giant-cell pneumonia of measles is classically described as an interstitial pneumonia, but the natural disease begins with a peribronchiolar localization of lesions that become diffuse. Significant pulmonary changes are alveolar and bronchiolar epithelial proliferation with typical giant-cell formation. Only a mild intraluminal serocellular exudate is present in the pure viral entity. In our experience, animals with this condition are susceptible to secondary bacterial invaders, and Diplococcus pneumoniae was frequently isolated.

MALLORY and MEDLAR [15], EWING [10], and, recently, SURINGA et al. [23] have described the skin lesions of measles in humans. BLAKE and TRASK [3] have done the same in monkeys. Only SURINGA et al. [23] have mentioned the epithelial syncytia that were so prominent in our skin sections. MALLORY and MEDLAR [15] illustrate syncytia in one of their figures (plate 14) and comment about the necrosis of epithelial cells, ‘... both singly and in small clumps’. According to BLAKE and TRASK [3] the measles rash of monkeys is essentially the same as the human counterpart. In this series we had only three monkeys in which epithelial syncytia were not observed in the skin. These three monkeys were biopsied late in the course of the rash. Although the number of monkeys sampled is small, the finding of epithelial syncytia in 15 of 18 monkeys indicates that the skin biopsy technique is a practical method to support a clinical diagnosis of measles in monkeys with a rash.

NII et al. [19] described multinucleated giant cells in the livers of monkeys infected with measles virus. The syncytia that they described were composed of lymphoid cells, undifferentiated hepatic lining cells, or Kupffer cells. The giant cells seen in 21 of 27 livers in our study were formed of hepatocytes.
The nuclear and cytoplasmic alterations of these syncytiia suggest a viral cause, but other commoner mechanisms such as hepatocellular regeneration must be considered [22].

The multifocal interstitial myocarditis observed in four monkeys was not associated with syncytial cells. The cardiac lesions were essentially the same as described for humans with the disease. In our experience mild interstitial myocarditis is a frequent finding in monkeys that die of various causes.

Measles lesions of the salivary gland are similar to those caused by cytomegalovirus infection. A characteristic microscopic feature of cytomegalovirus infection is periductal lymphocytic cuffing that is usually accompanied by intranuclear and cytoplasmic inclusions in enlarged ductal cells [6]. Most salivary syncytia seen with measles were not accompanied by inflammatory cells. It is not usually difficult to distinguish measles inclusions from cytomegalovirus inclusions but it may be difficult to differentiate the multinucleated cells associated with the virus of measles from those occasionally found with the cytomegalic virus. Mumps, which is also caused by a paramyxovirus, has been produced experimentally in monkeys [11]. It is not known to occur naturally in simians. Only cytoplasmic inclusions are found in the syncytia of mumps.

In our experience, multinucleated giant cells in the thyroid gland have been observed only in monkeys with measles. The presence of cytoplasmic and intranuclear inclusions in some of the syncytia in these monkeys further supports the contention that a virus causes the formation of these syncytia.

Other lesions associated with human measles but not detected in our material are those of subacute sclerosing panencephalitis [1, 27] and otitis media [2, 12].

References


Author's address: Major R. M. KOVATCH, Letterman Army Institute of Research, Presidio of San Francisco CA 94129 (USA)