Growth of Measles Virus in Epithelial and Lymphoid Tissues of Cynomolgus Monkeys

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The pathogenesis of measles virus infection is not yet clearly understood, but clinical and pathological findings as well as experimental studies in monkeys indicate the invasion of measles virus through the respiratory epithelium and initial multiplication in the regional lymphoid tissues spreading into the reticuloendothelial system, and subsequent infection of the epithelial tissues including the trachea and skin. Thus both the lymphoreticular cells and epithelial cells are major sites for measles virus replication (4).

In vitro growth of measles virus in the lymphoid cells has been used as the model of virus growth in these target cells in vivo (1, 2, 5). However, there is no report of a comparison of the in vivo growth of measles virus in epithelial and lymphoid cells. In the present study, characteristics of the growth of wild measles virus in the epithelial and lymphoid cells of cynomolgus monkeys (Macaca fascicularis) were compared by the immunofluorescence (IF) technique and electron microscopy.

Pooled throat washings obtained from several patients with measles a few days after the onset of rash were used as the source of wild measles virus as described previously (9). Two cynomolgus monkeys free of measles-virus neutralizing antibody were subcutaneously inoculated with the throat washings. Seven days later, they were euthanized by anesthetization with ketamine hydrochloride, and the brain, trachea, lungs, thymus, lymph nodes, spleen, kidneys, heart, and liver were removed. For the IF technique, the tissues were quickly frozen in n-hexane in a dry-ice-acetone bath. Thin sections made with a cryostat were fixed in acetone, and mixed with monoclonal antibodies against the Edmonston strain of measles virus including three clones against hemagglutinin (H) protein, one clone against nucleocapsid-associated phosphorylated (P) protein, one clone against nucleocapsid (NP) protein, two clones against fusion (F) proteins and two clones against membrane (M) protein (8), and then with goat anti-mouse IgG conjugated with fluorescein.

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1067
isothiocyanate. For histopathological examination, the tissues were fixed in 10% buffered formalin and embedded in paraffin. Paraffin sections were stained with hematoxylin and eosin (HE). For electron microscopy, the tissues were fixed in glutaraldehyde and osmium tetroxide, dehydrated in a graded alcohol series, and embedded in epoxy resin. Ultrathin sections were double-stained with uranyl
acetate and lead citrate, and examined under a JEOL 100 C electron microscope.

Both giant cells and virus antigens were found in the thymus, lymph nodes, spleen, and trachea, but not in the other organs. The giant cells, which consisted of both reticular and lymphoid cells (Fig. 1), were widely distributed in the lymphoid tissues, particularly in the germinal center. In the trachea, giant cells were found in the epithelium. These giant cells in the monkeys were histopathologically similar to those reported previously (7, 10, 13).

The synthesis of viral proteins in the trachea, spleen and mesenteric lymph nodes was compared by the indirect IF technique using monoclonal anti-measles virus antibodies. In the trachea, positive reactions were obtained with all the mono-

![Fig. 2. Viral antigen in the trachea (a, b, c, d) and spleen (e, f) detected by indirect IF.](image)

- a) anti-NP antibody (×200), b) anti-M antibody (×200), c) anti-H antibody (×200),
- d) anti-H antibody (×200), e) anti-NP antibody (×100), f) anti-M antibody (×200).
clonal antibodies tested. Antibodies to NP and P proteins strongly stained cytoplasmic material in the epithelial cells (Fig. 2a). Antibodies to M protein gave small dot-like fluorescence in the cytoplasm (Fig. 2b). Antibodies to H and F proteins stained the cytoplasmic membrane structures (Fig. 2c), but one monoclonal antibody (H-3) stained both intracytoplasmic material and cytoplasmic membrane structures (Fig. 2d).

In the spleen and lymph nodes, antibodies to NP and P proteins stained cytoplasmic materials in single cells and the syncytia with patterns varying from small dots to a confluent mass (Fig. 2e). Antibody to M protein gave small dot-like staining of cytoplasmic materials (Fig. 2f). In contrast, antibodies to H and F proteins stained neither single cells nor the syncytia.

By electron microscopy of the trachea, fuzzy nucleocapsids were detected in the cytoplasm and smooth nucleocapsids in the nucleus of the epithelial cells (Fig. 3a), and virus budding (Fig. 3b) as well as free virions were also detected. In the lymphoid tissue, fuzzy nucleocapsids were found in only the cytoplasm of giant cells (Fig. 3c), and neither virus budding nor free virions were detected.

In comparing virus growth in the epithelial and lymphoid cells, we found that
all five viral proteins (H, P, NP, F, M) were produced in the epithelial cells of the trachea, but production of H and F proteins was not detected in the spleen. These findings agree with those of Hyypia et al (2), who found that NP protein was produced in the peripheral blood mononuclear cells of measles patients, but H protein synthesis was restricted. Since the formation of giant cells in the lymphoid cells indicates the presence of functional F protein, F protein may be produced at a level undetectable by the IF technique or may be diffusely distributed.

It is also worth noting that electron microscopy revealed some differences in virus growth between the epithelial and lymphoid cells; the budding of virus and free viral particles were detected in the epithelial cells, but were absent in the lymphoid cells in spite of the presence of viral nucleocapsids. The failure to detect viral particles in the lymphoid tissues of measles-virus-infected monkeys in the presence of viral nucleocapsid was previously reported by Tajima and Kudow (12).

These findings suggest that replication of measles virus in the epithelial cells results in more efficient release of free virus than occurs in the lymphoid cells. Such restricted growth of measles virus in the lymphoid cells has been indicated by several investigators (2, 3, 6, 11). Whether the restricted production of H and F proteins in the lymphoid cells is relevant to the apparent lack of virus growth remains for future study. Efficient virus growth with release of free virus in the trachea seems to be teleologically significant in the excretion of virus for spreading infection to susceptible persons.

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