

LOCALIZATION OF A PR8 INFLUENZA VIRUS ANTIGENS IN TISSUES AND ORGANS OF MICE INFECTED INTRAPERITONEALLY AND INTRAVENOUSLY

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Summary. — Localization of A PR8 influenza virus antigens was studied by fluorescent antibody (FA) method in 50 mice infected intraperitoneally (ip) and intravenously (iv). Prior to its appearance in epithelial cells of lung and liver, virus antigen reproduction was observed in RES cells of lymph nodes, spleen, lungs and liver.

FA technique has frequently been used for the localization of influenza virus antigens in different cell systems. As shown by Mims (1960*b*), lung-adapted A PR8 virus, after iv infection of adult mice, is capable of multiplying in Browicz-Kupfer cells and hepatic cells and after intracerebral infection — in cells of the ventricular ependyma (Mims, 1960*a*). The present experiments were aimed at a determination of cellular localization of influenza virus antigens in mice infected ip and iv with special reference to the localization in cells of the reticuloendothelial system (RES).

Swiss outbred mice of both sexes, weighing 10—12 g, were infected with A PR8 influenza virus as follows:

50 mice ip with doses of 5×10^4 TCID₅₀, 15 mice ip with doses of 2×10^5 TCID₅₀ and 15 mice iv with doses of 5×10^4 TCID₅₀.

Frozen tissue blocks were stored at -20° C and cut in a kryostat. Tissue sections were fixed for 10 minutes in acetone at room temperature and then washed three times with buffered physiological saline, pH 7.6. FA staining was performed in a moist chamber at room temperature.

Rabbit immunoglobulins were conjugated with fluorescein isothiocyanate according to Riggs *et al.* (1958). The FA reagent thus obtained showed a haemagglutination inhibition (HI) titre of 1 : 1280 and protein concentration of 22.5 mg/ml. After absorption with rat liver acetone powder, the reagent was used diluted 1 : 4.

Localization of PR8 virus antigens in mice infected ip with 5×10^4 TCID₅₀.
Two hours after infection (p.i.) no viral antigens were detected in the organs. Six hours p.i. specific fluorescence was observed in the cytoplasm and nuclei of the macrophages in the marginal sinuses of the mediastinal lymph nodes, peripheral lymph nodes, in single reticulum cells surrounding lymphatic nodules of the spleen and in single Browicz-Kupfer cells of the liver. Small granules of the antigen material were also found in the walls of venous blood vessels in thymus, lungs and liver. Twelve and 24 hours p.i. the number of infected RES cells greatly increased. After 48 hours, the number of infected RES cells prominently decreased. After 96 and 144 hours influenza virus could not be found in the RES cells except of the cytoplasm and nuclei of single reticulum cells of the mediastinal lymph nodes. At that time large

amounts of influenza virus antigens were observed in lungs in the epithelial cells of bronchi and bronchioli and in single alveolar cells.

Localization of PR8 virus antigens in mice infected ip with 2×10^5 TCID₅₀.

Two hours p.i. of 2×10^5 TCID₅₀ inoculum was detected in the lymphatics of Glisson and thymic capsule and in the walls of venous blood vessels of thymus, liver and lungs. Six, 12 and 24 hours p.i., the amount of viral antigens in RES cells was much larger than that with the ip dose of 5×10^4 TCID₅₀ (Figs 1, 2). After 48 hours the amount of viral antigenic material in RES cells began to decrease and no viral antigens were observed in these cells after 96 and 144 hours, except of single reticulum cells in mediastinal lymph nodes. In lungs of mice that died after 96 hours the amounts of virus antigens were strikingly large especially in the cytoplasm and nuclei of epithelial cells in lung alveoli, small bronchi and bronchioli, in cells of the perivascular connective tissue and in the cells of the interalveolar septa (Figs 3, 4). In the multilayer cuboidal epithelium lining larger bronchi fluorescence of viral antigens was observed either in single cells of the basal layers or in the cytoplasm and nuclei of single cells of the superficial layer. Viral antigenic material released from infected cells undergoing desquamation or disintegration was found in cellular debris or in leukocytes of the exudate in the lumen of bronchi and alveoli (Figs 5, 6).

The data presented above suggest that after ip and iv injection of mice with PR8 virus the antigenic material is phagocytized by RES cells. The localization of PR8 virus in RES cells was similar to that reported by other authors for bacterial (Nossal *et al.*, 1964), viral (Mims, 1964) or protein (Mellors and Brzosko, 1962) antigens.

Gradual increase and decrease in the number of infected RES cells suggests virus multiplication in this system. Influenza virus was found in mediastinal lymph nodes after intranasal infection (Albrecht *et al.*, 1963; Liu, 1955) and in the spleen after iv infection (Mims, 1960*b*). These findings were interpreted by these authors as an evidence of phagocytic activity of RES cells. The present data concerning virus localization in the liver are essentially in accordance with findings reported by Mims (1960*b*). Viral antigens were detected in the cytoplasm and nuclei of Browicz-Kupfer cells and then in the hepatic cells; gradual increase followed by a decrease in the amount of antigenic material was also observed. The present findings confirm pathogenetic conclusions reported by Mims (1960*a, b*) who suggested that hepatic cells are infected by influenza virus just after its reproduction in Browicz-Kupfer cell. It should be mentioned, however, that the virus doses used in the present study were lower than the dose calculated by Mims as "toxic dose level" and considered necessary for "breakdown of the endothelial cell barrier" and for virus reproduction in these cells (Mims, 1960*a, b*).

As mentioned above, during the first 24–48 hours after parenteral infection viral antigens were found in the lungs mainly in RES cells. At that time virus introduced intranasally multiplies in respiratory and bronchial epithelium (Albrecht *et al.*, 1963; Denk and Kovac, 1965; Hers *et al.*, 1962). Differences in the cellular localization of PR8 influenza virus antigens during

the first 48 hours after parenteral and nasal inoculation seem to be explicable by different routes of infection.

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Explanations of Photomicrographs:

- Influenza virus antigens in mice intraperitoneally infected with 2×10^5 TCID₅₀ of A PR8 virus. Direct FA method.
- Fig. 1.* Viral antigens in the nuclei and cytoplasm of perifollicular macrophages in a mouse dead 24 hours p.i. $\times 360$.
- Fig. 2.* Viral antigens in the nuclei and cytoplasm of the hepatocytes in a mouse dead 24 hours p.i. $\times 500$.
- Fig. 3.* Viral antigens in the nuclei, some nucleoli and cytoplasm of endothelial, interstitial and epithelial cells in the lung from a mouse dead 96 hours p.i. $\times 225$.
- Fig. 4.* Brilliant fluorescence of viral antigens in the nuclei and cytoplasm of bronchial epithelium and in the peribronchial connective tissue from a mouse dead 96 hours p.i. $\times 225$.
- Fig. 5.* Viral antigens in the nuclei and cytoplasm of basal layers of bronchial epithelium mixed with cellular debris filling the lumen of a bronchus. Mouse killed 144 hours p.i. $\times 225$.
- Fig. 6.* Viral antigens in the cytoplasm of epithelial cells and in the lumen of a bronchial filled with cells debris. Mouse killed 144 hours p.i. $\times 225$.