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Preliminary Report

Studies on the Biological Effects of Live Attenuated Measles Vaccines in Monkeys

Since the development of live attenuated measles vaccines (Enders *et al.* 1960; Okuno *et al.* 1960; Smorodintsev *et al.* 1960) it has become necessary to establish reliable procedures for differentiating between epidemiological (wild) virus and attenuated vaccine virus. Marker tests to differentiate the attenuated Edmonston strain from the non-egg adapted strains were reported by Buynak *et al.* (1962) using tissue culture monolayers and these workers also recognized that the virulent strains produced severe lesions in the brain of monkeys when injected intrathalamically or intra-cisternally, while the attenuated vaccine virus did not cause such lesions.

It is well known that among the animals tested as hosts for experiments on measles virus, only the monkey suffers from a disease closely resembling human measles in both its clinical and pathological features (Blake *et al.* 1921; Shaffer *et al.* 1941; Taniguchi *et al.* 1954) and the monkey has been found to be easily infected naturally with human measles virus or MINIA (Ruckle, 1958). This high susceptibility to the agent make comparative experimental studies on the biological effects of wild and vaccine viruses possible in the monkey. Studies on the immunological and pathological responses of monkeys to measles vaccines are not only interesting, but necessary from a practical point of view.

This report describes preliminary experiments on this problem. In the preceding reports (Nii and Kamahora, 1963 a; Nii *et al.*, 1963 b) it was shown that viral specimens obtained from patients within 3 days after the onset of a rash as well as from those who showed Koplik's spots but no rash, caused extensive pathological changes in the bodies of monkeys or induced a definite antibody response in their sera, at appropriate times after virus inoculation. Indeed, all eighteen monkeys inoculated were affected by this material. Measles virus growth in the lymphatic tissues was confirmed by detection of intranuclear and cytoplasmic inclusions, as well as antigen in the cells of the tissues in addition to so-called Warthin-Finkeldey cells. However, it must be mentioned that the infective virus titers in the inoculum could not be determined, because usual virological assay procedures for epidemiological (wild) virus were very hard to make and this was a major problem in comparative experiments on vaccine and wild viruses.

Three series of experiments have been performed using live attenuated measles vaccines, which were egg-adapted and had been maintained by Dr. Okuno and his colleagues (1960) through serial egg transfers. Titration of the virus is possible on FL cell monolayers. The viral sample inoculated into monkeys was found to contain approximately 10^4-10^5 $TCID_{50}$ per ml. A half ml of this preparation

was inoculated subcutaneously into each monkey. In the first experiment the vaccines were inoculated into the right femoral area, while in the other two series of experiments they were inoculated into the right arm. Cynomolgus monkeys, weighing 1 - 2.5 kg, and originally obtained from Java, were used. Since it was possible that the monkeys had suffered naturally from measles before the experiments, vaccines were injected as soon as the animals arrived in this Institute (Nii and Kamahora, 1963 a; Nii *et al.*, 1963 b). Just before inoculation, blood was taken to see whether animals were immune to measles or not by the neutralization test or complement fixation test or by both. Just before death, blood was also taken to measure the level of the immunological response. Monkeys were killed by chloroform inhalation.

The general procedures used in these experiments are summarized in Table 1.

Table 1. Experimental Procedures Used to Study the Effect of Live Attenuated Vaccines in Cynomolgus Monkeys

no. of experimental series \ Days after inoculation	1	2	3	4	5	6	7	8	9	10	11
1			•(−)		•(−)		•(−)		•(−)		
2					•(−)			•(−)			
3				•(−)			•(+) •(±)				•(−)

• ..... signifies day when monkey killed
(−) ..... no specific pathological changes detectable
(+) ..... specific pathological changes found
virus..... live attenuated measles vaccine (Okuno *et al.*)

In each series of experiments monkeys were killed at appropriate times after injection of the vaccines.

In this series of experiments ten monkeys were used and of these two monkeys (Cases No. 46 and No. 47) showed pathological changes.

Both cases were in the third series of experiments and were killed 7 days after virus inoculation. In Case No. 47 the following pathological changes were observed: Formation of syncytia was observed in the epithelium of the bronchus. Nuclei in the syncytia were often found to be agglutinated and most of them had a central homogeneous substance with a marginal condensation of chromatin, suggesting full inclusion bodies. (Figs. 1 and 2) A moderate number of Warthin-Finkeldey cells were disseminated in the peribronchial lymph node and appeared to have rather small features. Cells were also occasionally found in other lymphatic tissues.

In Case No. 46 no definite characteristic changes could be seen in the respiratory tract. In the peribronchial lymph node typical Warthin-Finkeldey cells were rarely detected (Fig. 3) but there were a few scattered agglutinated cells with degenerated nuclei. A most peculiar pathological change in the lymph node in this case was a marked infiltration of macrophages into the lymph sinuses, especially the medullary sinuses. (Fig. 4) Some macrophages were found phagocytosing a variable number of cells resembling small lymphocytes. The cytoplasm of these cells was often hyperplastic and enlarged or swollen. In these macrophages, the nucleus could sometimes be discriminated from the many small nuclei derived from phagocytosed lymphocytelike cells, while the nucleus of macrophages was sometimes indistinct and so some polynuclear cells looked as if they consisted only of lymphocytes. (Figs. 5 and 6)

The phenomenon of lymphocytophagia by reticulum cells has been reported during poliomyelitis infection (Okano, 1956). As one of the geneses of Warthin-Finkeldey cells, the phagocytic action of macrophages was reported by both Bunting (1950) and Sherman *et al.* (1958). Histological findings on Case No. 46 undoutedly support this genesis, while a fusion of reticular cells such as is seen in tissue culture cells infected with measles virus should be considered as another genesis of giant cells in the lymphatic tissues.

No specific pathological changes were observed in other tissues and organs in either case. In the animals tested in the first and second series of experiments no definite specific pathological changes were found.

In the third series of experiments the immunological response of the monkeys was also tested and the monkey that was killed 11 days after virus inoculation showed a difinite rise in immunity. (Table 2)

Table 2. Correlation between Immunological Response of Cynomolgus Monkeys and Pathological Changes

Case number of monkey	Number of days from virus inoculation to sacrifice of monkeys	Neutralizing antibody titer		Specific pathological changes
		before virus inoculation	just before death	
45	4	<4	<4	−
46	7	<4	<4	±
47	7	<4	<4	+
49	11	<4	>64	−

Experimental series : No.3
virus : live attenuated measles vaccine (Okuno *et al.*)

So far as the above experiments are concerned, a low proportion of monkeys

(only two of ten monkeys) revealed pathological changes after inoculation of vaccine virus. This is in contrast with the severe affection of the animals by epidemiological virus. As all the animals were sacrificed at an earlier period after injection with the virus (between the 5th and 11th day), the mild effect of the agent on them could not be explained by a disappearance of the pathological changes due to recovery of the animals from the disease. The size of the inoculation dose might be another cause of the small effect of the vaccine virus, but a comparatively high dose of virus was used for the experiments.

Considering these facts, it may be said that the vaccine virus, which had been maintained by serial transfer in hatching eggs by Okuno *et al.* has less biological effect than wild virus and this indicates that it has attenuated pathogenicity.

Essentially, tissue tropism of the vaccine virus does not seem to differ from that of the epidemiological virus, as the former induced pathological changes in the epithelial cells of the respiratory tract as well as in the lymph nodes.

Hitherto, the existence of characteristic giant cells in the lymphatic tissues of host animals was thought to be a most reliable sign of infection. However, fluorescent antibody studies revealed many single cells containing viral antigen in the affected lymph node regardless of the existence of Warthin-Finkeldey cells (Nii and Kamahora; 1964). Therefore, absence of giant cells does not always imply that there is no viral growth in a given area. Similar studies by the fluorescent antibody technique in conjunction with routine histological observations might demonstrate more pathological features induced by live attenuated measles vaccine virus in monkeys.

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EXPLANATION OF FIGURES

Figs. 1 and 2. Syncytial formation in the bronchus. In the syncytia agglutinated nuclei have full inclusion bodies. Case No. 47 ×1000.

Fig. 3. A Warthin-Finkeldey cell in the peribronchial lymph node of Case No. 46 ×2500.

Fig. 4. Marked infiltration of macrophages in the medullary sinus of the peribronchial lymph node of Case No. 46 ×500.

Fig. 5. Macrophages in the lymph sinus of the peribronchial lymph node of Case No. 46, one of which is seen phogocytosing three lymphocytes. ×2500.

Fig. 6. The same preparation as shown in Fig. 5. One macrophage contains one small lymphocyte in the cytoplasm. One polynuclear cell, supposedly consisting of one macrophage and several phagocytosed lymphocytes, is also shown. ×2500.

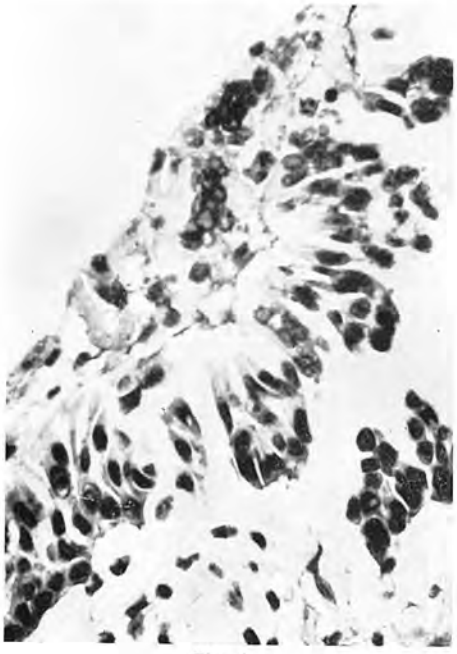

Fig. 1

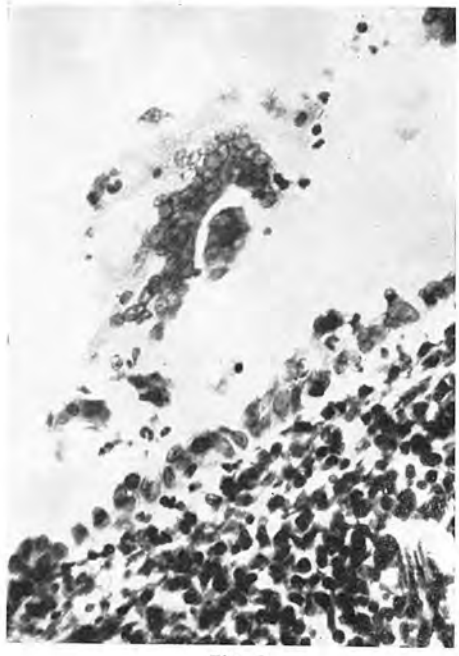

Fig. 2

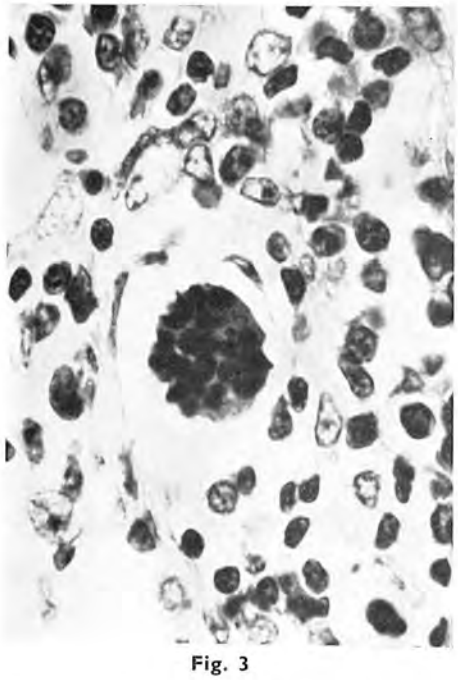

Fig. 3

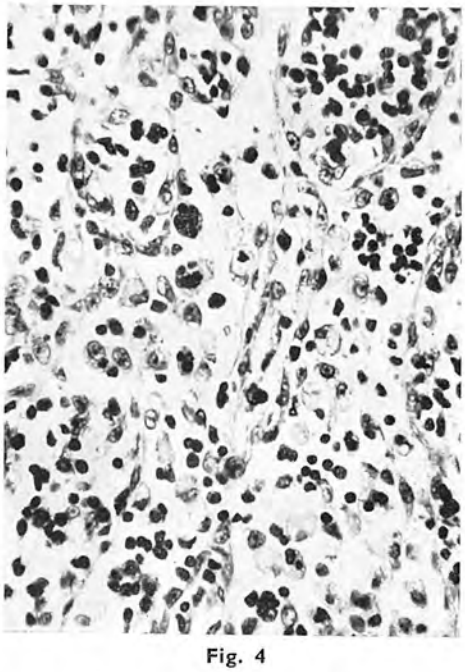

Fig. 4

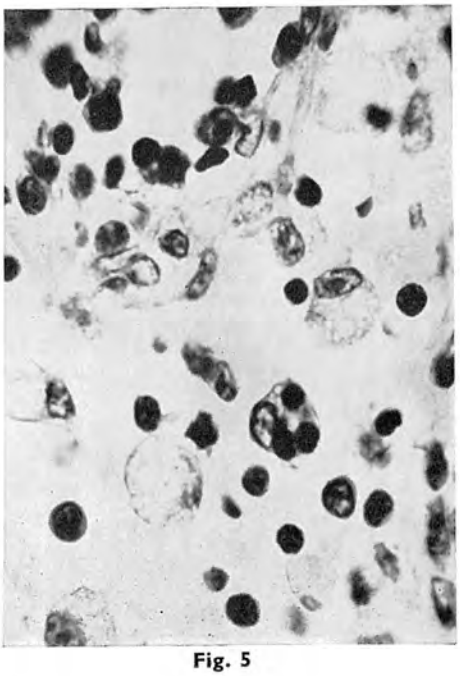

Fig. 5

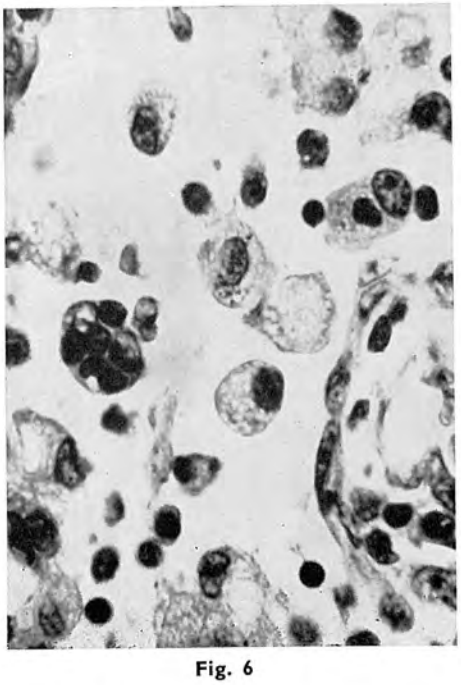

Fig. 6