

Inapparent Respiratory Infection of Inbred Swiss Mice with Sulfadiazine-Resistant, Iodine-Negative Chlamydiae

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Received for publication 4 June 1971

Three chlamydial strains were isolated from the lungs of three different strains of mice that were inbred for over 30 years. These chlamydial strains were resistant to sulfadiazine and did not induce iodine-positive, glycogen-like material in the inclusions of infected L cells which characterizes them as *Chlamydia psittaci*.

Inapparent respiratory infections of mice with chlamydial agents have been known since 1941 (4). This infection was subsequently identified in many stocks of mice used in biomedical research in all parts of the world. One of the murine chlamydial strains isolated by Nigg (8) in 1942 in the United States, representing five apparently identical chlamydial isolates from Swiss mice, was studied most intensively with regard to its specific chlamydial properties (9). The Nigg strain of murine chlamydial pneumonia induces an inclusion matrix which is iodine-positive, indicating glycogen-like material in the cytoplasmic inclusions, and its multiplication is inhibited by sulfadiazine (5, 10). These properties identify this strain as a representative of *Chlamydia trachomatis* which has always been associated with infections of man involving a strictly homologous chain of transmission (12). The Nigg strain of murine chlamydial pneumonia and the 12XN strain of hamster chlamydial pneumonia are the only known strains isolated from animals having such properties. The characteristics of three chlamydial strains isolated recently from the lungs of mice will be described in this note.

The strains Bab, S, and K of the mouse colony used for genetic research at Colorado State University were tested for respiratory chlamydial infection. These three strains of Swiss mice originated from different breeding stocks in the United States and Europe and were inbred for 25 to 38 years. Three-week-old mice from each strain were inoculated intranasally under light ether anaesthesia with 0.025 ml of suspensions prepared from lungs of several mice of various ages of the respective mouse strain. The inoculated mice of each strain were kept in separate cages. After a 7-day observation period, the mice were

killed, and suspensions of their lungs were inoculated into other 3-week-old mice of the respective mouse strains. After two or three lung passages in mice, the lung suspensions were inoculated by the yolk sac route into 7-day-old developing chicken embryos. The diluent was the sucrose-phosphate buffer of Bovarnick and co-workers (1) containing 500 mg of streptomycin per ml.

After the initial, second, and third intranasal passages, clinical pneumonic symptoms were not observed in inoculated mice, but small areas of consolidation and hyperemia were seen in the lungs of the second and third lung passages. Chlamydial elementary bodies were detected in Gimenez-stained (3) impression smears of some of these lungs. Chlamydial strains were isolated in developing chicken embryos from lung suspensions of the second or third passage material. These chlamydial isolates were identified as Bab, S, or K, according to the strain of inbred mice from which they were isolated. At least three passages in chicken embryos were required for each chlamydial isolate to induce death in chicken embryos. At all passage levels, chlamydial elementary bodies were detected in Gimenez-stained impression smears of yolk sacs of chicken embryos inoculated with low dilutions of the test material. After the third passage in chicken embryos, a regular death pattern was established. The average day of death was inversely related to the chlamydial concentration in the inoculum. This death pattern was similar for all three murine chlamydial strains. A dilution of 10^{-1} of the 4th passage killed chicken embryos after 96 hr, and embryos inoculated with the 10^{-7} dilution died after 245 hr. The average time of delay in death per decimal dilution of the K strain was 26 hr. The yolk sacs of the infected chicken embryos

were hyperemic, congested, and thin-walled, whereas the embryos were hyperemic and had cyanotic toes. Attempts to reisolate chlamydial agents from lung suspensions of the third mouse passage after 2 months of storage at -20°C were successful with the K and Bab strains. Boiled suspensions of infected yolk sacs of all three isolates fixed complement in the presence of a serum dilution containing 2 units of antibodies against group-specific chlamydial antigen.

The sensitivity to sulfadiazine of our murine chlamydial isolates and the Nigg strain of murine chlamydial pneumonia was tested by using the method of Page (10). The infectivity of the Bab, K, and S strains was not reduced in the presence of 1 mg of sulfadiazine per chicken embryo receiving 0.5 ml of decimal chlamydial dilutions (Table 1). The infectivity of the Nigg strain was reduced by more than 6 \log_{10} (11).

Cover slip-grown L cells were infected with partially purified suspensions of our murine chlamydial strains and the Nigg strain. Cells were stained with iodine by the method of Gordon and Quan (5) or by the Giemsa method 24, 36, 48, and 60 hr after infection. Inclusions in cells infected with Bab, K, and S strains did not react in the iodine stain, but positive iodine reactions were seen in inclusions of cells infected with the Nigg strain. Cell preparations stained by the Giemsa method 36 hr after inoculation with all four murine strains had purple, round, and compact chlamydial inclusions in the cytoplasm. The inclusions had variable sizes and shapes at 48 and 60 hr after inoculation, and those associated with the Bab, K, and S strains became more diffuse and granular. Some of the infected cells contained more than one chlamydial inclusion.

The biological properties of the chlamydial strains Bab, K, and S, which were recently isolated from lungs of mice, clearly characterize them as strains of the species *C. psittaci*. These strains differ from the Nigg strain which has properties of *C. trachomatis*. However, Weiss and co-workers (14) found that deoxyribonucleic acid (DNA) of this chlamydial strain only formed thermolabile reassociations with the DNA of strains of *C. trachomatis* derived from human infections. Gerloff and Watson (2) found two other chlamydial strains from mice having properties similar to our strains and compatible with *C. psittaci*. The Nigg strain should, therefore, not be considered as a prototype for respiratory chlamydial infections of mice (7). It is evident that different chlamydial species are associated with infections of laboratory mice, which are similar to chlamydial infections in man. In this connection, it is of

TABLE 1. Sulfadiazine sensitivity of different murine chlamydial strains and iodine reaction of their cytoplasmic inclusions

Chlamydial strain	-Log ₁₀ CELD ₅₀ titer		Iodine reaction of inclusions
	Control	Sulfadiazine (2 mg/ml)	
Bab.....	8.50	8.50	Negative
K.....	8.16	8.16	Negative
S.....	7.83	8.16	Negative
MoPn (Nigg).....	8.33	<2.0	Positive

interest that strains of *C. trachomatis* derived from human ocular infections were adapted experimentally to multiply in the lungs of mice despite the high host specificity of this chlamydial species (6, 13).

This investigation was supported by Public Health Service research grants AI 08420 and AI 07399 from the National Institute of Allergy and Infectious Diseases.

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