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Cilia injury during virus infection in chickens

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SUMMARY

The reduction of mucociliary clearance in the Newcastle disease virus-B infected nasal turbinate in chickens is mainly due to ciliary pathology, decrease of the number of cilia and of the ciliary beat frequency.

Common cold alters the nasal physiology. This alteration is very important, because there is extension of the inflammation to the nasopharynx, pharynx, sinuses, ears as well as to the lower respiratory tract. According to a Japanese proverb: thousands of diseases originate from common cold.

The chicken has been chosen as the experimental animal for the virus infection of the upper respiratory tract. According to Bang and Bang (1959), the chicken has relatively simple turbinates, true sinuses, a mucociliary, acinar respiratory epithelium and less developed olfactory systems than other small laboratory animals such as the mouse, rat or rabbit. The nose of man and chicken are quite similar in their structures and tissue systems.

In chickens, intranasal inoculation of the Newcastle disease virus B strain produces the mild inflammation, which is located in the inner lining of the maxillary turbinate. There are no systemic involvements nor an influence on the maxillary sinuses. Damaged epithelium completely recovers within three weeks (Bang and Bang, 1977). They show that the NDV-B infected nose in chicken is analogous to an acute rhinitis in man caused by common cold.

It has been shown that common cold depresses the mucociliary clearance in the nose, but the reports are not quite in agreement on the extent of the depression. Quinlan et al. (1969) reported that mucociliary clearance was absent in two patients with acute upper respiratory infection, whereas two other patients had normal transit rates. Sakakura et al. (1973) showed on experimentally induced rhinovirus infection induced a decrease in the transport rate of particles. The maximum effect was on the third day after inoculation. From the twelfth day on,

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there was no significant decrease in particle transport. Andersen et al. (1977) reported that a rhinovirus infection caused a gradual decrease of the flow rate of nasal mucus starting two days after the virus instillation, and after five days the rate was less than half its initial value. Simon et al. (1977) reported that no mucociliary transport could be demonstrated in five out of nine subjects with a common cold ten days before or one week after the investigation. Ginzel and Illum (1981) reported that in case of a naturally acquired common cold, two subjects had a normal clearance, in 12 subjects nasal clearance returned to normal within three weeks and in five subjects normal clearance reappeared after five to eight weeks.

The mechanism of decreasing mucociliary clearance induced by common cold has not been fully understood. It is certainly not so simple that only mucosal damage is responsible for the slowness. There are two contradictory opinions: the first states that there is only a minor change in the epithelium (Winther et al., 1984) and the other argues that there is a massive destruction and prolonged regeneration of the nasal epithelium in naturally acquired common cold (Pedersen et al., 1983).

Using the NDV-B infected nose in chicken, we have endeavoured to clarify the relationship between mucociliary clearance and cilia injury during acute viral infection in the nose.

MATERIAL AND METHODS

Commercially obtained Babcock chickens, 21 days old, were inoculated with Newcastle disease virus B strain. Undiluted allantoic fluid from stocks of B strain of NDV was used for intranasal inoculation. To induce different degrees of infection, three different amounts of virus were inoculated: 1. one drop of 10^{-1} titer of $10^{7.5-9.0}ELD_{50}$ per 0.05 ml; 2. one drop of 1 titer of $10^{7.5-9.0}ELD_{50}$ per 0.05 ml, and 3. three drops of 1 titer of $10^{7.5-9.0}ELD_{50}$ per 0.05 ml.

Histopathological examination

The nasal turbinates of the chicken were removed: one was used for light microscopic examination with H and E, and the other for scanning electron microscopy. Histopathological findings were divided into five grades: 0. no significant change; 1. slight changes of the epithelium including acinal glands; 2. mild and local infiltration of inflammatory cells; 3. selective but severe inflammatory changes of the inner mucosa and 4. severe inflammatory changes of all the inner mucosa.

The histopathological gradation was compared with the mucociliary clearance and the ciliary area.

Mucociliary clearance

A great advantage of using chickens is that their mucociliary clearance can be measured non-invasively and repeatedly through a cleft of the palate. The chickens were placed in a plastic holder and the mouth was held open with an adjustable device. Ten µl of food dye was placed in each nostril, and the time from this moment until the dye appeared at the choanal margin was measured with a stopwatch under an operating microscope (Wakabayashi et al., 1977; Ukai et al., 1983).

Ciliary area

The term "ciliary area" implies the area which is occupied by cilia on the surface of the epithelium. The ciliary area is expressed in a percentage which reflects the ratio of a ciliary area in a given area. The ciliary area was measured by a quantitative scanning electron microscopy. The place to measure a ciliary area was the inner surface of the maxillary turbinate. This inner surface which was about 0.4 mm^2 was photographed serially by SEM. Using an image analyzer, the ciliary area which had been encircled by hand in a picture were automatically added up (Figure 1).

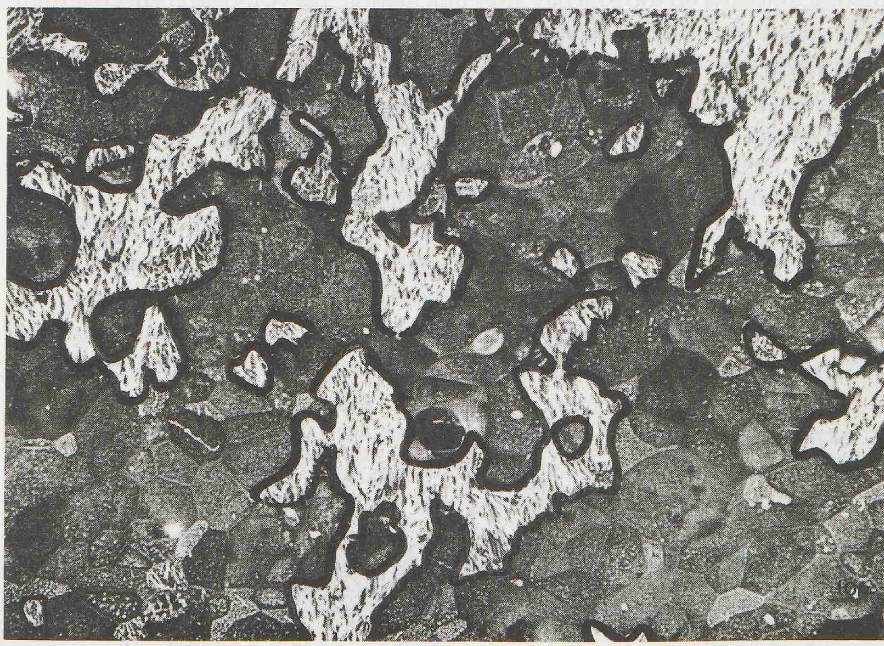

Figure 1. Ciliary area was encircled for an image analyzation. The ciliary area of this scanning electron micrograph is 31.6%. Focal nature of loss of cilia is observed. There is no denudation. Non-ciliated cells with various microvillous projections replace the normal ciliated surface.

Ciliary beat frequency

The photoelectric method was used for the measurement of ciliary beat frequency. In this pilot experiment, one drop of 1 titer was inoculated and the ciliary beat frequency of 30 different cells from one turbinate was measured.

Virus recovery

Pharyngeal swabs were used for virus isolation. Samples were tested by direct inoculation of the chorionic membrane of 10 or 11 day-old chicken embryos. The result was expressed by ELD_{50}.

Serum HAI antibody

When an experiment was finished, the chickens were killed by cardiac bleeding. Using a microtitre kit, haemaglutinin inhibition titrations were performed.

RESULTS

All inoculated chickens showed positive immunologic evidence of infection. The least amount of inoculated virus showed the least response of serum antibodies on the 7th day. However, the level of antibody response was the same for three different amounts on the 21st day after inoculation. No virus was recovered from throat swabs after 5 days of infection.

Mean transit time of the mucociliary clearance was 100.6 sec + 37.6(SD) (N = 15) in the control group. NDV infection reduced the mucociliary clearance in the nose of chickens. A proportional reduction of the clearance to the amounts of in-

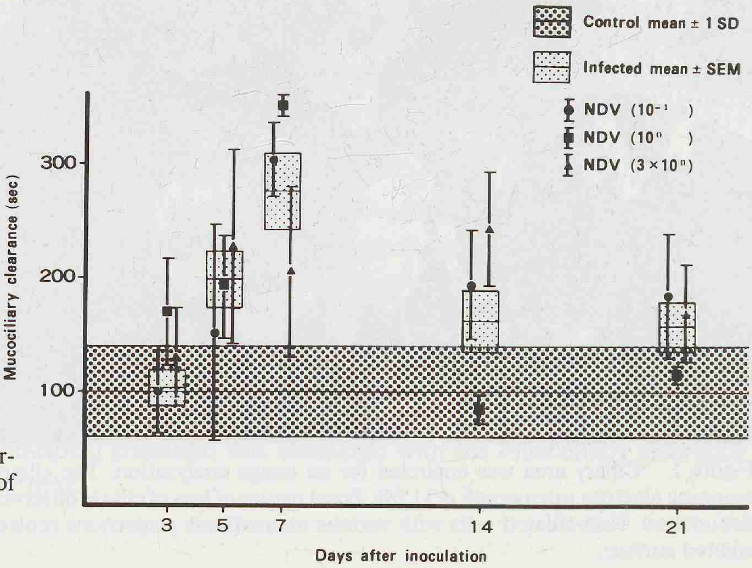

Figure 2. Effect of different amounts of NDV-B on mucociliary clearance.

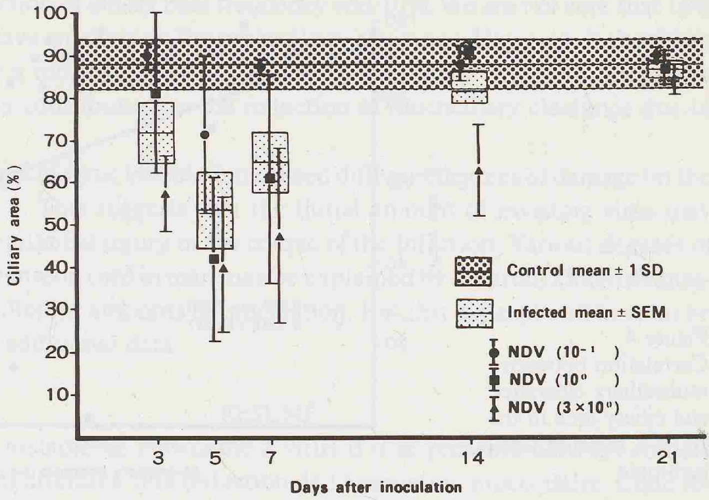

Figure 3.
Effect of different amounts of NDV-B on ciliary area.

oculated virus was not observed. The peak of reduction of the infected group was on the 7th day and the mean transit time was 274 sec. On the 21st day, mucociliary clearance was still reduced in this experiment (Figure 2).

Figure 3 shows the change of ciliary area after virus exposure. The mean ciliary area in the control group was 88.3% + 5.8 (SD) (N = 15). Three drops of NDV caused the severest damage on the surface of the turbinate. In proportion as the amount of inoculated virus increases, the ciliary area will decrease.

The ciliary area was reduced to 70% in the minimum inoculated chicken on the 5th day. After the 5th day it returned to normal. The ciliary area was reduced to 40% in the maximum inoculated chickens on the 5th day, and was still reduced to 62% on the 14th day, and finally returned to normal on the 21st day.

In Figure 1, the ciliary area occupied 31.6% of the surface. The most striking feature of this NDV infection was a loss of cilia. Desquamation was never observed. Microvillous projections replace the normal cilitated surface.

A significant inverse correlation between ciliary area and mucociliary clearance was established in the NDV-B infected turbinate in chickens. Ciliary area decreases with increased mucociliary clearance time (Figure 4). From Figure 4, if ciliary area decreases less than 60%, the mucociliary clearance time will exceed 280 sec which is about three times of the mean clearance time of the control group. However, in an individual chicken, there was no correlation between mucociliary clearance and ciliary area.

Ciliary beat frequency of the control chickens averaged 14.0Hz + 2.6(SD). Although the number of tested chickens was small, all infected chickens (N = 9), except one, showed a significantly reduced beat frequency.

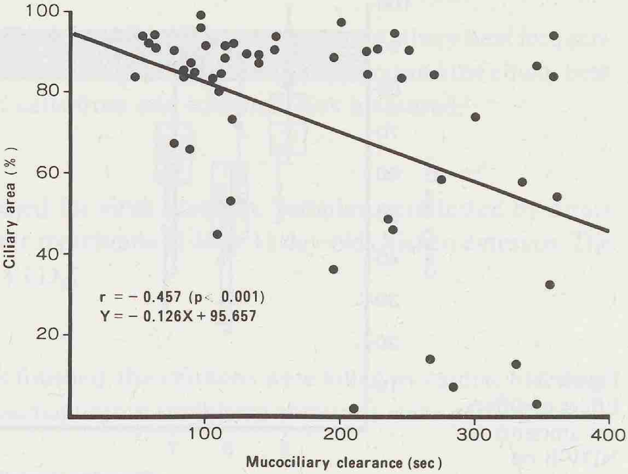

Figure 4.
Correlation between mucociliary clearance and ciliary area in the NDV-B infected nasal turbinate

DISCUSSION

The total area measuring cilia was about 0.4 mm^2. It is questionable whether or not this small area can represent the pathology of the entire turbinate. In order to clarify this question, the ciliary area was compared with the histopathological gradation in light microscopic findings. An inverse correlation between histopathologic gradation and ciliary area was found. However, the correlation between histopathological gradation and mucociliary clearance was less significant. Because we know and examine the exact place on the turbinate, which shows the maximum change, our method for the evaluation of the ciliary area is satisfactory. However, no information is available about common cold in man and, therefore, we do not know the representative place in the nose.

In chickens the most striking feature of the surface of the infected epithelial surface was focal loss of cilia. It was not clear whether only cilia or ciliated cells had been destroyed. However, no denudation was noted. Denudation of the nasal epithelium was observed in tracheobronchitis virus infection in chickens (Bang and Bang, 1967) and in influenza A virus infection in ferrets (Chevance et al., 1978). There was a general inverse correlation between the ciliary area and mucociliary clearance as to time. First, the ciliary area began to recover five days after inoculation, and after 7 days the mucociliary clearance began to recover. A significant inverse correlation between ciliary area and mucociliary clearance was found in the NDV-B infected turbinate in chickens. However, in 20% of the infected chickens this correlation was not found. Changes in rheological properties of mucus and/or malinteraction of cilia and mucus would be the explanation for this (Majima et al., 1983; Sakakura, 1983).

The mean reduction of ciliary beat frequency was 10%. We are not sure that 10% reduction will have an affect on the mucociliary clearance. However, it should be considered that a reduction of the ciliary beat frequency might be one of the causative factors contributing to the reduction of mucociliary clearance due to virus infection.

Different amounts of virus inoculation caused different degrees of damage on the epithelial surface. This suggests that the initial amount of invading virus may determine the epithelial injury in the course of the infection. Various degrees of complexity in common cold in man may be explained by different kinds of causative virus and different amounts of inoculation. But this concept will have to be supported with additional data.

RÉSUMÉ

Au cours de la maladie de Newcastle à virus B il se présente dans les cornets nasaux de poules atteintes, une réduction de l'évacuation mucocilaire. Cette réduction est due principalement à une pathologie ciliaire; de même, une diminution du nombre des cils et de la fréquence de battement ciliaire.

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