Acta virol. **36**: 152-156, 1992

VIRUS-SPECIFIC IMMUNE RESPONSE IN THE LUNGS OF MICE INFECTED WITH INFLUENZA VIRUS

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Received April, 1989; revised February 4, 1991

Summary. - The time course of primary humoral immune response in NFS/N mice infected with the adapted influenza virus A/Aichi 2/68(H3N2) was followed by determination of the different class immunoglobulins in lungs, lung washings, and in blood serum. The quantity of antibody-producing cells (APC) was estimated by local haemolysis in gel. The presence of antibodies in serum and lavage fluid was tested by the methods of radial haemolysis and radial immunodiffusion. It was shown that the local immune response had developed earlier than systemic antibodies occurred in the serum.

Key words: influenza virus; mice; antibodies

Introduction

The significance of humoral immunity in acute respiratory infections was shown in numerous publications. It is known that the resistance of lung tissue to infection is in a considerable degree stimulated by local antibody production (Rossen *et al.*, 1971; Waldmann *et al.*, 1973). However, the development of humoral immune response in the human respiratory tract during influenza virus infection or post vaccination has been insufficiently studied. Several authors (Waldmann *et al.*, 1968; Butler *et al.*, 1970; Murphy *et al.*, 1982) found virus-neutralizing antibodies in human tracheobronchial secretion obtained at bronchoscopy and/or autopsy and the alteration in total counts of cells secreting antibodies into the lavage fluid. The presence of virus-specific antibodies and cells, producing antibodies in the lungs and bronchoalveolar washings of mice, infected with different types of influenza virus has been described by several authors (Scott and Sydiskis, 1976; McLaren *et al.*, 1978; Jones and Ada, 1987).

The aim of the present work was to follow the local development of virus-specific APC, which secrete immunoglobulins of different classes into the lungs of infected animals and to determine the role of local antibody secretion

as related to systemic immune response to influenza virus.

Materials and Methods

Experimental animals. Eight to twelve week old mice of NFS/N strain were obtained from the nurseries of the USSR Academy of Medical Sciences. The animals were kept in plastic cages with light day duration of 12 hr. Five animals (12–14 weeks old) of the same age and sex were used in each experiment.

Virus strains. We used A/Aichi 2/68(H3N2) strain of influenza virus which was adapted to lung tissue of mice. This strain was propagated in the allantoic cavity of the 10–11-day-old chicken embryos and incubated for 40–48 hr. Virus-containing allantoic fluid was stored at -70 °C.

Experimental infection. Influenza virus was inoculated by intranasal route under ether anaesthesia in a 50 μl of virus-containing allantoic fluid (diluted with cooled Hanks solution) in a dose, causing the death of 50–70 % of mice ($1LD_{50}$).

Suspension of mice lung cells was prepared as follows: the lungs were placed in Petri dishes containing 5 ml of RPMI-1640 medium (Serva), supplemented with 10 % of heated foetal calf serum (Serva), 0.05 ml sodium pyruvate (Serva), 0.05 ml amino acids solution (Serva), 0.05 g L-glutamine (Serva), 100 units/ml of penicillin and 50 μg of streptomycin. The lungs were cut to fragments 3 x 3 mm and incubated in a 0.1 % solution of *Clostridium histolyticum* (Fluka) during 30 min at a room temperature. Then the material was run several times through a 0.5 mm plastic tube. The mononuclear cells suspension was obtained by gradient centrifugation on Ficoll-Hipaque (Pharmacia) and subsequent washing with the RPMI-1640 medium.

The number of APC was determined by the method of Jerne and Nordin (1963) in the modification of McLaren *et al.* (1978). Briefly: sheep red blood cells after 3-week storage at 0 °C were incubated for 15 min at room temperature with corresponding virus dilution in the presence of 5×10^{-4} mol potassium periodate. The working dilution of the virus was determined in radial haemolysis in gel, using sheep red cells, covered with the virus and anti-influenza virus A/Aichi 2/68(H3N2) serum of mice. Red cells were washed in Hank's solution and resuspended in Veronal-buffered saline. The agarose with low temperature of gel formation (Type V11, Sigma) was used. The determination of APC secreting immunoglobulins of different classes was carried out by the method of Gronovics *et al.* (1976), using monospecific antisera against heavy chains of mouse class M, G, A immunoglobulins.

Peripheral blood. The samples were obtained from retro-orbital plexus of anaesthesized animals. After clotting the serum has separated, clarified by centrifugation and stored at -20 °C.

Lung washings. The animals were killed by cervical spine dislocation. The lungs were removed and thoroughly washed with Hanks solution. Perfusion was carried out through the right atrium in order to reduce blood contamination of the lung washings. Then the warm Hanks solution was administered into trachea and after a careful massage of the lungs the washings were aspirated. The procedure was repeated several times. The total volume of the washings was 3 ml. The obtained samples were purified by centrifugation for 20 min at 3 000 rev/min at 4 °C. The supernatant was stored at -20 °C.

The presence of antibodies in the serum of peripheral blood and lung washings was tested by radial haemolysis in gel (Russel *et al.*, 1975); the immunoglobulins of different classes were detected by radial immunodiffusion according to Mancini *et al.* (1963).

Results and Discussion

It was established that in the lungs of healthy intact mice there are cells, interacting non-specifically with influenza virus. Their number was low (11.3±2.5

background APC per 10^6 nucleous containing cells) and did not reach the limits of statistical significance. The development of influenza infection was accompanied by proliferation of bronchial lymphoid tissue. The number of APC secreting immunoglobulins of different classes increased considerably during the acute period of infection. A significant amount of IgM-APC was detected already two days following virus inoculation (38.4±4.5 per 10^6 mononuclear cells). The number of IGM-APC reached its peak 6 days p.i. The peak of IgG-APC took place on day 8 after infection, but changes in their quantity started on the 5th day of follow-up; at this time 64.2±6.3 (x 10^6) APC was detected. The development of influenza virus infection of mice caused the appearance of considerable number of IgA-APC, their quantity was relatively high throughout the whole investigation period (Fig. 1). The primary immune response to influenza virus, as judged by the occurence of APC, was accompanied by the appearance of virus-specific antibodies in the serum and lung washings (Fig. 2). Detection of virus-specific antibodies, belonging to different classes, in the serum of experimental animals showed that on day 14 p.i. only IgM and IgG could be identified (precipitation zones measured 7.3±0.2 mm and 7.1±0.6 mm, respectively). Some decrease in the amount of IgM and subsequent increase of IgG in the serum was detected on day 21 p.i. (precipitation zones 11.8±2.4 mm and 12.7±1.3 mm, respectively).

We failed to determine the class-specificity of viral antibodies in the lung washings. Apparently, this can be explained by the relatively low sensitivity of the method, since Barbar and Small (1978) and Zee *et al.* (1979) succeeded in the detection of IgM, IgG, and IgA in lungs of experimental animals using other methods and after preliminary concentration of the lavage fluid.

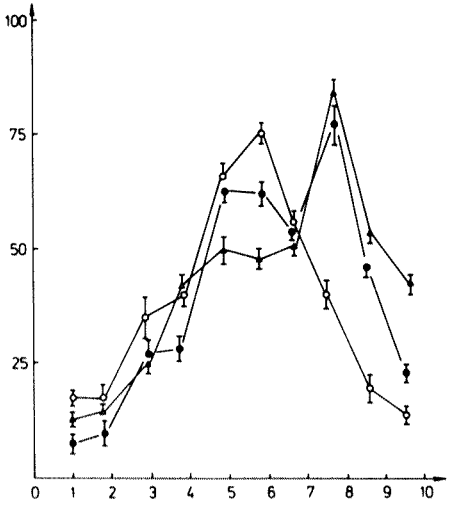

Fig. 1
The primary virus-specific immune response in the lungs of the mice infected with influenza virus. (○) - IgM-APC; (●) - IgG-APC; (△) - IgA-APC; each point corresponds to the results as detected in five animals
Abscissa: the time after virus inoculation (days). Ordinate: the number of APC/10^6 mononuclear cells

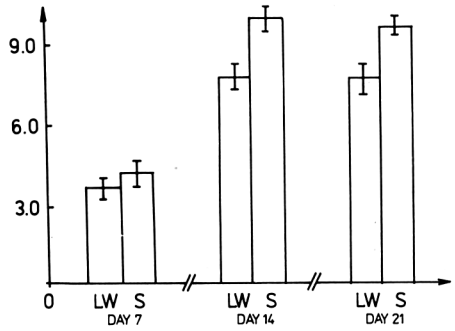

Fig. 2
Virus-specific primary immune response in the serum (S) and lung washings (LW) in infected mice; each point corresponds to the results as detected in five animals Abscissa: the time after virus inoculation (days). Ordinate: the square of haemolysis areas (mm^2). Results in the serum and lung washings of intact mice were negative.

The kinetics of local immune response development in the target organ in viral infections, which are characterized by week dissemination of the agent, is of special importance. Scott and Walker (1976) characterized the development of immune response in the respiratory system by the change of the quantity of cell population containing membrane-associated immunoglobulins; these authors failed to identify virus-specific APC. Jones and Ada (1987) demonstrated the presence of a considerable amount of similar cells, producing virus-specific antibodies of different classes with the help of enzyme immunoassay.

The lesser number of virus-specific APC, revealed by us in the lungs of infected animals can be related to lesser sensitivity of local haemolysis. The different course of the isotypical response development can be explained by the difference in the doses of virus used. In our opinion, the higher dose of the virus stipulated a longer presence of infectious agent in the respiratory tract. However, we succeeded to demonstrate the coincidence of IgG and IgA response in the course of antibody formation in lungs.

The data obtained characterize the development of humoral immune response to influenza virus in lungs of mice in acute period of infection. Local immune response develops upon the presence of infectious agent in a respiratory tract and can be detected prior to serum antibodies. It is obvious that virus-specific antibodies produced locally react first with the virus promoting its removal from the body.

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