DIAGNOSTIC VALUE OF THE OXYPHILIC INCLUSIONS IN INFLUENZA

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The dynamics of the number of oxyphilic inclusions (OI) and the titer of influenza antigen (IA) was investigated in experiments on 350 albino mice infected with A2 (Hong Kong) 68 influenza virus. The accumulation of IA in the trachea took place parallel with the appearance of OI. IA appeared before OI in the cells of the alveolar epithelium, especially in animals infected with average doses of the virus. The biphasic dynamics of its discovery in the respiratory organs characteristic of this strain of influenza virus correlates exactly in time with the 2 peaks of IA activity in the trachea on the 4th day and in the lungs on the 7th-8th day after infection. The discovery of IA, like the detection of OI in the affected lung tissue, is of diagnostic importance, for it reflects the dynamics of the viral infection.

KEY WORDS: oxyphilic inclusions; influenza antigen; alveolar epithelium.

Oxyphilic (fuchsinophilic) inclusions (OI) are constantly found in the cytoplasm of the epithelium of the respiratory tract in acute viral respiratory diseases [1, 2, 6, 9-11]. Their morphology has been described in particular detail in experimental influenza [3-5, 7]. These cytoplasmic inclusions are a reflection of the infectious process connected with propagation of the virus, and for that reason their discovery is of diagnostic importance. Meanwhile no quantitative determination of OI has yet been carried out in different stages of the disease, nor has the existence of correlation between their appearance and the accumulation of the virus been investigated.

The investigation described below was carried out to study this problem.

EXPERIMENTAL METHOD

A2 (Hong Kong) 68 virus, after 3 passages in chick embryos, was adapted for 15 passages to the lungs of albino mice, after which it gave rise to infection in these animals followed by their death, depending on the dose of the virus, on the 5th-10th day after administration. In the experiments the mice were infected intranasally with doses of 10, 100, and 1000 LD_{50}. The accumulation of the virus in the lungs was determined by titrating a lung suspension in chick embryos. Accumulation of influenza antigen (IA) in the cells was determined (per 100 cells) by examination of films from the trachea and lung in the luminescent microscope by Coon's method and the number of OI was counted. Films were taken 3 and 6 h after infection and then daily for 9 days. To compare the number of cells containing IA and OI in the course of the influenzal infection, the method of regression analysis was used [8].

EXPERIMENTAL RESULTS

In the first experiment the mice were infected with 10 LD_{50} of the virus (Fig. 1). The animals died on the 6th-9th day after infection. The content of virus in the mouse lungs reached its maximum on the 4th and 7th days after infection.

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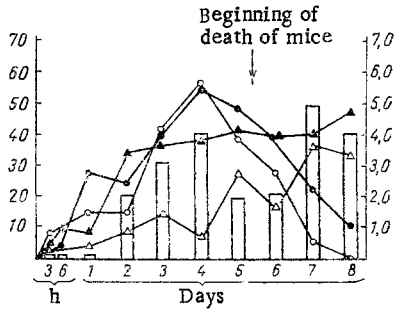

Fig. 1

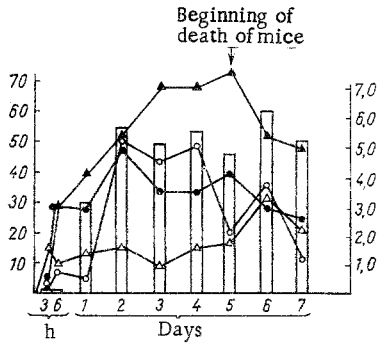

Fig. 2

Fig. 1. Correlation between virus activity in the lungs of infected mice and dynamics of the OI and IA in epithelial cells of the trachea and lungs (infecting dose of virus 10 LD_{50}). Empty circles and triangles represent inclusions in the trachea and lungs respectively. Columns show quantity of virus in lungs. Filled circles and triangles show antigen in the trachea and lungs respectively. Abscissa, time after infection of animals; ordinate: right – quantity of virus (LD_{50}) in respiratory organs, left – percentage of cells containing OI or IA.

Fig. 2. Correlation between virus activity in lungs of infected mice and dynamics of OI and IA in the epithelial cells of the trachea and lungs (infecting dose 100 LD_{50}). Legend as in Fig. 1.

In the first 3-6 h, single IA granules were found arranged around the nucleus in individual cells of the tracheal epithelium. At the same time tiny, solitary OI appeared. By the end of the first day many cells with granular cytoplasm and a large hyperchromic nucleus, containing IA and tiny OI, were seen in the ciliated epithelium. Later degenerative changes took place in the tracheal epithelium. Many of the cells were vacuolated, some severely. The nuclei of these cells were large, as a rule pale, but occasionally hyperchromic, and fragmented; between the vacuoles in the cytoplasm numerous fairly large OI appeared. In the first 4 days after infection the number of cells with intracellular OI correlated with the accumulation of the virus antigen. The number of OI in the trachea reached a maximum on the 4th day (55%). At the same time the highest number of cells containing IA (57%) was observed in the trachea. Its content in each individual cell increased and the granules often fused together to form large droplets, or they were scattered diffusely throughout the cytoplasm. Later there was a gradual decrease in the number of cells with IA and OI or they disappeared completely.

The alveolar epithelium showed no distinct changes during the first 2 days. Meanwhile only individual cells containing IA and OI were seen in films from the lungs. However, by the end of the 2nd day the number of cells with specific luminescence increased to 34%, much more than the number of cells with OI (9%). The number of cells with IA remained high until the end of the observation, increasing gradually to 47%; this correlated with the quantity of virus isolated from the lungs. The number of cells containing OI also increased, although not so regularly – to 37% by the 7th day. Just as in the tracheal epithelium, considerable morphological changes took place in the alveolar epithelium. The cells in which OI were found were hypertrophied. Their nuclei appeared pale or, less frequently, hyperchromic, and they were sometimes displaced toward the periphery. At times the cell cytoplasm was granular, but later it became vacuolated. Specific luminescence in the alveolar epithelium was at first finely granular, and located chiefly in the perinuclear zone, but by the 5th-7th day the granules had joined into larger drops. The OI initially also was small and single, but as the influenza progressed they became larger and more numerous.

In the second experiment a larger dose of virus (100 LD_{50}; Fig. 2) was used. In this case the animals began to die on the 5th-7th day and the virus accumulated more quickly in the lungs.

The morphological changes in the cells containing OI were the same as in the previous experiments. IA was found for the first time in the ciliated epithelium 6 h after infection in a fairly high percentage (29%) of the epithelial cells. OI were discovered much more rarely. The accumulation of IA and OI later took place parallel to the increase in virus activity. From the 2nd to the 4th day IA was found in 49% of cells and OI in 49-53% of cells. Later the number of cells containing IA (25-30%) and OI (20-33%) decreased.

TABLE 1. Character of Propagation of A2 (Hong Kong) 68 Virus in Lungs of Albino Mice

Dose of virus (in LD_{50})	Dynamics of virus activity			Epithelial cells with OI				Epithelial cells with IA			
	phase of viral propagation	Activity of virus (in LD_{50})	time of discovery (days)	regression coefficient	time of discovery (days)	percent of cells with OI	regression coefficient	location	time of discovery (days)	% of cells with IA	regression coefficient B
100	Rise	5.5	2	Trachea	2	53	+25.2±4.9	Trachea	2	48	+18.7±6.1
	Fall	4.7	5	"	5	20.5	−7.3±4.5	"	7	25	−3.3±1.0
	Rise	6.2	6	Lungs	6	33	+1.4±0.7	Lungs	5	76	+13.0±5
10	Rise	4.0	4	Trachea	4	57	+11.9±4.5	Trachea	4	55	+11.6±11.2
	Fall	2.0	5	"	5—7	28	−13.5±1.2	"	8	12	−11.2±2.4
	Rise	5.0	7	Lungs	7	37	+3.9±1.3	Lungs	4—8	42	+4.4±2.0

Regression coefficient B reflects the degree of increase (+) or decrease (−) in the percentage of affected cells.

In this experiment, by contrast with the first, specific luminescence in the cells of the alveolar epithelium was observed during the first few hours after infection, simultaneously with the appearance of IA in the tracheal epithelium. The number of cells containing OI was much smaller, not more than 10-20%. With the course of time this difference increased still more: on the 1st day 40 and 13.5%, on the 2nd day 54 and 16%, and on the 3rd and 5th days the maximal virus activity was observed and this was accompanied by the largest number of cells of the alveolar epithelium containing IA (70-75%). By the end of the period of observation (7th day) a tendency for the number of epithelial cells containing IA to decrease began to appear (49%). The number of alveolar epithelial cells containing OI did not exceed 20% during the first 5 days, and not until the 6th day was their number increased to 33.5%.

In the 3rd experiment an even larger dose of virus (1000 LD_{50}) was used. Under these conditions the animals died during the first 5 days after infection. The pathological changes in the epithelium in the trachea and lungs observed under the light microscope were similar to those in the previous experiments. IA was found initially in the tracheal epithelium: after 3-6 h luminescence was observed in individual cells and a peak was reached 24 h after infection with the virus (14%). Later the number of infected cells remained fairly constant, then gradually fell to 5% after 5 days. The increase in the number of cells with OI was rather slower; the first inclusions were found after 12 h (1%), the number reached a maximum (9-10%) on the 2nd-4th day, and thereafter it fell parallel to the decrease in IA. The curve of the increase in number of alveolar epithelial cells with specific luminescence in the films from the lungs was less steep than that for the tracheal epithelium. It reached its maximum by the time of the animals' death (11%). OI appeared in the alveolar epithelium 12 h later than the specific luminescence (by the end of the 1st day). The curve of their accumulation in the cells was similar in form to the curve of accumulation of IA in the cells, but there were fewer cells with OI than with IA.

The results of these experiments indicate different relations between the accumulation of virus antigen and the appearance of inclusions in films from the lungs and trachea. After infection of the animals with 10 and 100 LD_{50} of virus, the accumulation of IA in the trachea took place parallel with the appearance of OI, as reflected by closely similar values of the regression coefficients (Table 1) for antigen and inclusions (25.2 and 18.7 for a dose of 100 LD_{50} and 11.9 and 11.6 for a dose of 10 LD_{50}). IA appeared sooner than OI in the cells of the alveolar epithelium; this was particularly evident after infection with 100 LD_{50} of the virus, when the difference between the maximal number of cells with inclusions and antigen (33 and 76%) is confirmed by the corresponding regression coefficient, namely 1.4 and 13.0. For the small dose of virus definite correlation was found between the dynamics of appearance of IA and OI in the trachea and lungs and the dynamics of the content of active virus, determined by virological methods. The diffuse character of propagation of this strain of virus in the respiratory organs showed clear correlation with the peaks of activity of the virus antigen in the trachea on the 4th day and in the lungs on the 7th-8th day after infection.

When the average dose of virus (100 LD_{50}) was used there was no clear bimodal curve of accumulation of virus activity, evidently because of the rapid dissemination of virus multiplying in the trachea and in the lungs. This was confirmed by the discovery of many (up to 70%) alveolar epithelial cells containing IA during the first 4-5 days after infection. OI, however, were found at this time in a very small percentage of cells.

The discovery not only of IA, but also of OI is thus of diagnostic importance for it shed light on the dynamics of the viral infection.

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