EFFECT OF VIRUS-NEUTRALIZING ANTIBODIES ON INTRA- AND POSTREPRODUCTIVE SPREAD OF ADENOVIRUS INFECTION

M. B. Maksimovich and S. P. Lisovaya

UDC 616.988.5-085.373-039.17

Investigations with adenoviruses of types 4 and 6 showed that the infectious agent (the factor of intrareproductive spread), which is present only in freshly removed culture fluid and is sensitive to deoxyribonuclease (DNase), is not neutralized by antibodies against the corresponding viruses, as shown by inhibition but not arrest of the infectious process in tissue culture during growth of the adenoviruses in medium with an excess of homologous antibodies. Additional treatment with DNase under certain conditions leads to resolution of the infectious process.

A previous investigation [1] showed that the infectivity of the liquid phase of cell cultures infected with adenoviruses is attributable to two factors. The first, unstable, was inactivated by deoxyribonuclease (DNase). The second (mature virus particles) was resistant to this enzyme. The unstable factor migrated from the infected cells into the liquid phase 10 h sooner than the mature virus particles, i.e., before they had completed reproduction, and for this reason the spread of infection brought about by this factor was called intrareproductive, to distinguish it from the postreproductive spread due to mature virus particles. It would be more correct to regard the infective factors described above as precursors of virus particles rather than as infectious DNA, as was suggested previously.

In the investigation described below the possibility of neutralizing the factor of intrareproductive spread by means of antivirus antibodies was studied.

EXPERIMENTAL METHOD

The decanted freshly extracted liquid phase of FL cells taken 48 h after infection with type 6 adenovirus with a multiplicity of 1 TCD_{50}/300 cells was poured into four tubes. Twice the neutralizing dose of antiserum was added to the first tube containing fresh material and the stored mother virus with equivalent infectivity, DNase (50 μg/ml with 0.04 M Mg^{++}) was added to the second tube, antiserum and DNase in the same quantities were added to the third tube, and the corresponding volume of medium no. 199 to the fourth tube (control). The samples were incubated for 1 h, after which the infectious activity was determined cytomorphologically [2].

To study the effect of antibodies and of DNase on the development of the infectious process, type 4 adenovirus was cultivated in a low multiplicity of infection (0.2 $TCD_{50}/10^5$ FL cells) in medium containing the previous doses of DNase or antibodies or both together, and also in their absence (control). The results were read 48, 72, 96, 120, and 144 h after infection, also using a cytomorphological criterion.

Adenoviruses of types 4 and 6 with titers of $10^{3.02}$ and $10^{2.07}$ TCD_{50}/ml (read on the 3rd day), antisera neutralizing 1 TCD_{50} of these viruses in dilutions of 1:320 and 1:1280 respectively, and DNase with an activity of 25-30% of the crystalline substance were used in the investigation.

I. I. Mechnikov Odessa Research Institute of Virology and Epidemiology. (Presented by Academician of the Academy of Medical Sciences of the USSR V. D. Solov'ev.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 74, No. 9, pp. 77-79, September, 1972. Original article submitted September 21, 1971.

TABLE 1. Results of Neutralization of Infectivity of Freshly Isolated and Stored Type 6 Adenovirus

Character of treatment	Number of infected cells (per 1000 from the monolayer) after infection of tissue culture*	
	with freshly isolated virus	with stored mother virus
Control (untreated)	552, 559, 581, 534, 501 $M \pm m = 545{,}4 \pm 14{,}9$	652, 571, 588
Neutralization with antiserum	94, 142, 146, 126, 14 $M \pm m = 104{,}4 \pm 27{,}1$	0, 0, 0
Treatment with antiserum and DNase	0, 4, 0, 0, 2, $M \pm m = 1{,}2 \pm 0{,}8$	—
Treatment with DNase	303, 287, 312, 291, 281 $M \pm m = 299{,}8 \pm 10{,}3$	—

*In individual observations.

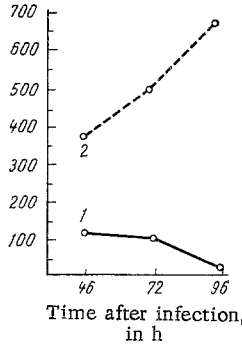

Fig. 1. Dynamics of infection with type 4 adenovirus in medium with DNase and antibodies with renewal of the latter every 24 h: 1) experiment; 2) control. Abscissa, times of observation (in h) after infection; ordinate, number of cells with adenovirus inclusions per 1000 cells from monolayer.

EXPERIMENTAL RESULTS

The results of neutralization of the freshly isolated and stored type 6 adenovirus are given in Table 1. Infection of the cell culture with untreated fresh and with stored material produced lesions in 545 and 652 of 1000 cells respectively, corresponding to a concentration of about 1 TCD_{50} of virus in the infecting dose. Treatment of this dose with a double excess of antibodies led to complete neutralization of the stored virus and to the preservation of residual infectivity in the fresh material, which produced lesions in 104.4 ± 27.1 (in some cases over 140) of 1000 cells. The combined action of antibodies and DNase in all cases completely or almost completely suppressed the infectivity of the freshly isolated material, while the action of DNase alone merely reduced it.

A twofold excess of antibodies thus did not abolish but merely reduced the infectivity of the fresh material, even though it is known that addition of only one equivalent of antibodies is sufficient to produce neutralization when added to the virus particle [3, 8], and an excess of antibodies completely neutralizes the adenovirus [5]. Inactivation of the residual infectivity by DNase implies that this inactivation cannot be the result of the action of monovalent antibodies [4, 6, 7], dissociation of the virus–antibody complex [5, 9], or separation of virus aggregations, for in those cases mature virus particles resistant to the nuclease would be liberated. Consequently, the residual infectivity was due to infectious agents insensitive to the antibodies. Their inactivation by DNase confirms that these are identical with the factor of intrareproductive spread.

In the course of cultivation of type 4 adenovirus in medium no. 199 (control) with antibodies alone or with DNase alone the number of infected cells increased to 674.4 ±23.9, 317.9 ± 16.4, and 324 ± 36.1 respectively. During the combined action of antibodies and DNase (Fig. 1) with renewal of the antibodies every 24 h, the number of infected cells did not increase during the course of infection, as in the control, but fell significantly ($P < 0.01$) from 115.5 ± 10.0 at the beginning of observation to 31.2 ± 4.8 at its end (results of 42 observations).

As a result of neutralization of the mature virus particles (blocking of postreproductive spread) the antibodies inhibit but do not arrest the infection. Under these conditions infection develops through intrareproductive spread, on which the antibodies have no action. Further treatment with DNase destroys the factor of intrareproductive spread and leads to resolution of the infectious process.

The factor of intrareproductive spread is absent in stored virus, so that the use of this material for the titration of antisera does not provide a reliable criterion suitable for determination of their preventive properties in serotherapy.

LITERATURE CITED

1. M. B. Maksimovich, in: Influenza and Acute Respiratory Diseases [in Russian], Part 2, Leningrad (1967), p. 232.
2. M. B. Maksimovich and M. S. Parfenova, Vopr. Virosol., No. 6, 694 (1968).
3. R. A. Hawkes and K. J. Lafferty, Virology, 33, 250 (1967).
4. N. K. Jerne and P. Avengo, J. Immunol., 76, 200 (1956).
5. L. Kjellen, Virology, 18, 448 (1962).
6. K. J. Lafferty, Virology, 21, 61 (1963).
7. K. J. Lafferty, Virology, 21, 76 (1963).
8. H. Rubin, Virology, 6, 533 (1957).
9. C. Todd, Brit. J. Exp. Path., 9, 244 (1928).
10. D. A. Tyrrell, J. Exp. Med., 97, 863 (1953).