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A SIMPLE AND RAPID CHARACTERIZATION OF INFLUENZA VIRUS ISOLATES BY MONOCLONAL ANTIBODIES IN RADIOIMMUNOASSAY

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Summary. — Radioimmunoassay (RIA) with infectious allantoic fluid directly bound to solid phase, suitable for detection and further characterization of influenza virus isolates, is described. This simple and rapid method was applied for description of isolates obtained from different regions of Czechoslovakia during influenza epidemic in 1983. The results confirmed that all 13 examined isolates represent influenza A viruses possessing H3 subtype haemagglutinin very similar to haemagglutinin of influenza viruses A/Bangkok/1/79 (H3N2), A/Belgium/2/81 (H3N2) and A/Philippines/2/82 (H3N2), respectively.

Key words: influenza virus isolates; detection; characterization; solid phase RIA, monoclonal antibodies

A rapid diagnosis of influenza virus infections can be made now by detecting viral antigens directly in nasopharyngeal specimens with highly sensitive and specific techniques such as RIA and ELISA (Sarkkinen *et al.*, 1981; Evans and Olson, 1982). Definitive description of influenza virus strain(s) responsible for the perspective epidemic requires, however, also the isolation of the virus and an additional testing of the isolated viruses at present usually performed by haemagglutination-inhibition or neuraminidase-inhibition tests and immunodiffusion test using standard antisera.

The purpose of this work was to show that influenza viruses can be detected and characterized in detail using RIA with allantoic fluid directly bound to solid phase. Allantoic fluids from inoculated chick embryos are analysed without any pretreatment or partial purification of virus. Using this method and panel of 7 monoclonal antibodies, we characterized viruses isolated in different regions of Czechoslovakia during the influenza epidemic in 1983.

Antigens. We have examined the samples of allantoic fluids from chick embryos inoculated with virus isolates which underwent, after successful isolation, less than 4 (usually 2—3) passages in chick embryos. Samples were analysed directly, i.e. without any pretreatment or partial purification of virus. As a control infectious allantoic fluids containing following standard virus strains have been used: A/Bangkok/1/79 (H3N2), A/Belgium/2/81 (H3N2), A/Philippines/2/82 (H3N2), A/Singapore/1/57 (H2N2), A/England/403/80 (H1N1), A/PR/8/34 (H1N1).

Table 1. Characterization of influenza A virus isolates in SP-RIA

Virus strain	No. passages[1])	HAU per ml	Gu-3[2]) anti-NP	Gu-3[2]) anti-HA			Gu-2[3]) anti-HA		
			IVE8	IIB4	IIF5	IIE5	IVG6	IVA1	IIF4
A/Praha/1/83	E_4	512	4.4[4])	5.4	6.5	7.5	4.0	4.5	3.0
A/Praha/2/83	E_4	128	3.4	5.5	7.4	6.9	3.5	3.0	2.5
A/Praha/3/83	E_xE_1	512	3.2	3.9	7.4	5.9	3.9	2.4	3.4
A/Bratislava/1/83	E_6	128	4.0	6.3	7.6	6.1	4.1	4.6	3.1
A/Bratislava/9/83	E_3	256	5.4	4.0	10.9	8.4	4.4	4.4	4.4
A/Bratislava/12/83	E_2	512	6.0	7.6	9.6	7.6	5.1	5.1	3.6
A/Bratislava/19/83	E_xE_1	128	5.6	3.2	5.1	4.0	1.6	1.6	1.1
A/Bratislava/51/83	E_xE_1	512	1.9	3.5	6.2	6.7	4.2	3.7	2.7
A/B. Bystrica/65/83	E_4	256	2.6	4.1	5.7	5.7	2.2	2.2	1.7
A/B. Bystrica/114/83	E_2	1024	3.7	3.8	5.0	6.0	2.5	2.5	2.0
A/B. Bystrica/130/83	E_2	256	3.0	3.4	4.5	4.5	2.5	2.5	1.5
A/B. Bystrica/164/83	E_2	512	4.0	3.3	7.6	6.6	4.6	4.1	2.6
A/Hradec Králové/1/83	E_xE_1	256	3.7	3.0	4.5	4.0	1.5	1.5	1.3
A/Bangkok/1/79 (H3N2)		512	2.1	2.5	2.2	2.2	1.2	1.2	0.7
A/Belgium/2/81 (H3N2)		256	5.7	2.8	5.2	3.7	2.7	3.2	2.2
A/Philippines/2/82 (H3N2)		1024	6.0	3.6	5.5	4.5	2.0	2.5	2.0
A/Singapore/1/57 (H2N2)		1024	0.1	0.1	0.1	0.1	0.1	0.0	0.3
A/England/403/80 (H1N1)		512	1.3	0.1	0.0	0.0	0.1	0.0	0.2
A/PR/8/34 (H1N1)		1048	1.4	0.0	0.1	0.1	0.0	0.0	0.1

[1]) in chick embryos; the first passage was intraamniotic, further intraallantoic. E_x — number of passages unknown.
[2]) obtained after immunization with A/Bangkok/1/79 (H3N2)
[3]) obtained after immunization with A/Dunedin/4/73 (H3N2)
[4]) cpm $\times 10^3$. Appropriate corrections were made for instrument background and for the radioactivity non-specifically bound to antigens (cca 400 cpm) after incubation with the medium from Sp2/0 cells

Monoclonal antibodies. Six monoclonal antibodies against haemagglutinin (IIB4-IgG2a, IIE5-IgG2a, IIE5-IgG2a, IVG6-IgG1, IVA1-IgG2a, IIF4-IgG1) and one monoclonal antibody against nucleocapsid protein (IVE8-IgG2a) were produced by hybridomas obtained after fusion of the mouse myeloma cell line Sp2/0 with spleen cells from mice immunized with influenza virus A/Dunedin/4/73 (H3N2) or A/Bangkok/1/79 (H3N2). The production and characterization of these monoclonal antibodies will be described in more detail elsewhere (manuscript in preparation).

Solid phase RIA. Undiluted allantoic fluid (25 μl) was added to individual wells of 96-well polystyrene plates (KOH-I-NOOR, Czechoslovakia) and dried overnight at 37 °C. Dried samples were saturated 1 hr with bovine serum albumin, washed, incubated 2 hr with monoclonal antibody (diluted ascitic fluid giving 50% of the maximum radioactivity which can be bound to 150 ng of purified influenza virus A/Bangkok under the same experimental conditions), washed, incubated for 2 hr with ^{125}I-labelled rabbit anti-mouse F(ab')$_2$, washed, and counted in gamma counter. As a negative control medium from Sp2/0 myeloma cells was used. Iodination of rabbit anti-mouse F(ab')$_2$ was done as described (Russ *et al.*, 1978).

Influenza virus isolates (13) and standard influenza virus strains belonging to different subtypes have been analysed in solid phase RIA with 7 monoclonal antibodies (Table 1). First conclusion which can be made from the results is that influenza viruses can be detected using RIA with allantoic fluid directly bound to solid phase, and that this way of detection has satisfactory sensitivity and specificity. Binding of type specific IVE8 monoclonal antibody (directed against nucleocapsid protein) was significant to all 13 examined isolates as well as to standard influenza virus strains of H1, H2 and H3 subtypes. From this result we can therefore conclude that all 1983 examined isolates were type A influenza viruses.

Further characterization of virus isolates was possible with monoclonal antibodies against haemagglutinin. Binding of these monoclonal antibodies to virus isolates and control viruses of H1, H2 and H3 subtypes confirmed that all new virus isolates can be classified as H3. Finally, fine specificity of anti-haemagglutinin monoclonal antibodies used in the present study (described elsewhere — Russ *et al.*, in preparation) confirmed that all 1983 examined isolates are very similar to influenza virus A/Bangkok/1/79 (H3N2), A/Belgium/2/81 (H3N2) and A/Philippines/2/82 (H3N2) respectively (Masár, 1984; Walter, 1984).

Although there were rather large differences among individual isolates in the level of reactions (which reflects with high probability just the different content of viruses in allantoic fluids) the reactivity pattern of all virus isolates with our panel of 7 monoclonal antibodies was essentially the same, i.e. no differences were detected among individual isolates with these monoclonal antibodies in solid phase RIA. In HI-test, however, one out of seven our monoclonal antibodies, namely IIB4, detected significant differences. Certain isolates (e.g. A/Praha/1/83; A/Bratislava/12/83; etc.) gave as high as 1 : 20,000 HI-titres with IIB4 monoclonal antibody as long as other isolates (e.g. A/Bratislava/1/83; A/B. Bystrica/130/83; etc.) were completely negative in HI-test with this monoclonal antibody. These results are described and discussed in accompanying paper (Styk *et al.*, 1986).

RIA with allantoic fluid in solid phase seems to be suitable metod for simple and rapid detection and further characterization of isolated influenza viruses. In principle the solid phase RIA is the binding test. Therefore it

is possible to use also such monoclonal antibodies which cannot be used in biological test where their capacity to inhibit particular biological activity is required. This enabled us to use our anti-haemagglutinin monoclonal antibodies in described experiments, since with exception of IIB4 monoclonal antibody, these monoclonal antibodies are negative in HI-test under standard conditions. Finally, the preciseness, reproducibility and large capacity of solid phase RIA is well known.

There is no doubt that application of monoclonal antibodies in solid phase RIA analysis of isolates in allantoic fluids is advantageous. The background reaction with monoclonal antibodies is negligible, so the assay is highly specific and sensitive. With application of a large and appropriate panel of monoclonal antibodies it would be possible to obtain a detailed characterization of viruses very soon after their isolation.

Polyclonal antisera against virus or isolated envelope glycoproteins contain also antibodies against host antigen showing cross-reactivities with all viruses. It is necessary (often not easy) to remove these non-specific antibodies by adsorption. Monospecific polyclonal antisera against internal proteins do not contain any anti-host antigen antibodies, since they are type specific, they can provide just limited informations. It is apparently advantageous to use monoclonal antibodies in highly sensitive assays such as RIA or ELISA. We assume, however, that antisera containing a mixture of polyclonal antibodies cannot be in the near future totally replaced with monoclonal antibodies. Polyclonal antisera can provide, in addition to monoclonal antibodies, further and essential informations (for example in biological tests).

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