Monoclonal Antibodies for Rapid, Strain-Specific Identification of Influenza Virus Isolates

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Monoclonal antibodies conjugated with fluorescein permitted rapid, strain-specific identification of influenza A isolates and type-specific identification of influenza B isolates by direct immunofluorescence staining. Identification of H1 influenza A strains could be accomplished by direct immunofluorescence on cell culture fluids lacking sufficient hemagglutinin activity to permit identification by hemagglutination inhibition.

Influenza viruses were among the first to which monoclonal antibodies were applied for more definitive antigenic analyses, and these immune reagents proved to be extremely sensitive tools for antigenic mapping of viruses and for defining antigenic drifts (3, 7-9).

In efforts to speed and facilitate the identification of influenza virus isolates, monoclonal antibodies to recent influenza A and B strains were produced and examined for suitability in direct immunofluorescence (DIF) staining for strain-specific identification of current and recent viral isolates.

Viruses used for antibody production were the A/Berkeley/1/80 H3N2 strain (closely related to the A/Bangkok/1/79 strain by hemagglutination inhibition [HI] testing), the A/USSR/90/77 H1N1 strain, and the B/Singapore/222/79 strain. These were concentrated 10-fold from infected allantoic fluids by adsorption to and elution from chicken erythrocytes. BALB/c mice 6 to 8 weeks of age were immunized by the following schedule. On days 0 and 7 the animals received 0.2 ml of a mixture of virus concentrate and an equal volume of Freund complete adjuvant by the intraperitoneal route; on day 21, virus concentrate with Freund incomplete adjuvant was given in 0.2-ml volumes subcutaneously into each of three sites; and on day 35, 0.2 ml of virus concentrate was given intravenously. After 3 to 4 days the spleens were removed for fusion.

The Sp 2/0-Ag14 line of mouse myeloma cells (6), kindly provided by M. B. A. Oldstone, Scripps Clinic and Research Foundation, La Jolla, Calif., was used for fusion with the immune spleen cells. Cell fusion was done with polyethylene glycol 1540 by published procedures (4). Fluids from the hybrid cell cultures were screened for influenza viral antibody by

indirect immunoperoxidase staining of virus-infected cynomolgus monkey kidney cells, using peroxidase-labeled anti-mouse immunoglobulin G produced in rabbits (Miles Laboratories, Elkhart, Ind.) and aminoethyl carbazole as a substrate. Antibody-positive cell populations were cloned two times by the limiting dilution method and then inoculated intraperitoneally into pristane-treated BALB/c mice of 3 to 6 months of age at a concentration of 4×10^6 cells. Immune ascitic fluids from the mice constituted the working monoclonal antibody preparations.

Serological reactivities of the immune ascitic fluids selected for use in these studies are shown in Table 1. HI tests were performed by standard procedures (1), and the indirect immunoperoxidase (5) and indirect immunofluorescence (2) methods have also been described. The three reagents reacted to varying titers with homologous viruses, and showed no reactivity with heterologous ones. By indirect immunoperoxidase staining the H3N2 reagent reacted with the homologous strain and the A/Bangkok/1/79 strain, but failed to react with the earlier H3N2 strains, A/Hong Kong/5/72 and A/Texas/1/77. The influenza B reagent showed no HI activity, but high type-specific titers by indirect immunoperoxidase and indirect immunofluorescence, indicating that it was directed against a type-specific viral component rather than the hemagglutinin. None of the monoclonal antibody preparations showed complement-fixing activity with homologous antigens.

For virus identification by DIF staining, the monoclonal antibodies in immune ascitic fluids were labeled with fluorescein isothiocyanate (2), and the conjugates were titrated against monkey kidney cells infected with homologous and heterologous viruses to determine the optimal

TABLE 1. Serological reactivity of monoclonal antibodies to influenza viruses

Immune ascitic fluid	Test system ^a	Antibody titer of immune ascitic fluid against:		
		A/USSR/90/77 H1N1	A/Berkeley/1/80 H3N2	B/Singapore/222/79
A/USSR/90/77 (XVIII-E-G-7-3)	HI	128	<8	<8
	IIP	≥2,048	<8	<8
	IIF	≥128,000	<8	<8
A/Berkeley/1/80 (IXX-C-15-2-2)	HI	<8	≥2,048	<8
	IIP	<8	≥2,048	<8
	IIF	<8	≥128,000	<8
B/Singapore/222/79 (XXII-D-5-12-3)	HI	<8	<8	<8
	IIP	<8	<8	≥2,048
	IIF	<8	<8	≥128,000

^a IIP, Indirect immunoperoxidase; IIF, indirect immunofluorescence.

working dilutions. All three conjugates were used at working dilutions of 1:300, and they showed no reactivity at dilutions as low as 1:8 with heterologous viruses. Standard conjugates for influenza A and B viruses, which showed type-specific reactivity by DIF, were prepared from antisera produced in hamsters (1) and were used at working dilutions of 1:15.

Influenza virus isolation attempts were performed in tube cultures of primary cynomolgus monkey kidney cells (1). Because our isolates from the 1981–82 season consisted solely of H1 and influenza B strains, some specimens from the 1980–81 season which had yielded H3 influenza A isolates were reinoculated into cell culture and examined with monoclonal antibody conjugates. As soon as the cultures showed a cytopathic effect or hemadsorption suggestive of infection with influenza virus, the cells were scraped into the fluids, sedimented by low-speed centrifugation, and suspended in 0.03 ml of phosphate-buffered saline with 2% fetal bovine serum to prepare smears for DIF. Smears were air-dried, fixed in acetone, stained with working dilutions of conjugates for 20 min at 35°C, and then examined with a Zeiss fluorescence microscope with incident light and ×250 magnification. Each isolate was examined against monoclonal antibody conjugates to the H1N1 and H3N2 strains of influenza A and to B/Singapore/222/79, and against standard, type-reactive conjugates for influenza A and B viruses.

Identification was based upon staining with one of the strain-specific influenza A monoclonal antibody conjugates and the standard influenza A conjugate, or with the monoclonal and standard influenza B conjugates. In the initial phase of this study, confirmatory identification was done by HI with antisera prepared in hamsters. DIF staining with the monoclonal antibody conjugates clearly identified the isolates as H1 or H3 strains of influenza A, or as influenza

B viruses (Table 2). Identification by DIF was accomplished on day 3 to 5 after inoculation for the H1 strains, at day 3 for the H3 strains, and on days 3 to 5 for the influenza B strains. DIF was particularly useful for identification of the H1 influenza A isolates, since these strains had low hemagglutinin titers in the cell culture fluids, often requiring additional passages before identification could be accomplished by HI. Even then, cell culture fluids frequently contained fewer than 4 hemagglutinating units optimal for use in an HI test.

The ability to perform strain-specific identification of influenza virus isolates by more rapid and simpler methods than HI is very useful for epidemiological surveillance in the current situation, where influenza A H1N1 and H3N2 strains

TABLE 2. Identification of influenza virus isolates with conjugates of monoclonal antibodies

Conjugate	No. of isolates positive by DIF	Identity of positive isolates by HI
A/USSR/90/77, H1N1	22	H1N1, 8 of 8 tested
A/Berkeley/1/80, H3N2	9	H3N2, 9
Type-reactive ^a influenza A, A/Texas/1/77	31 ^b	H1N1, 8; H3N2, 9
B/Singapore/222/79	8	Influenza B, 3 of 3 tested
Type-reactive ^a influenza B, B/Hong Kong/5/72	8	Influenza B, 3 of 3 tested

^a Standard antisera produced in hamsters.

^b Isolates which reacted positively with H1 or H3 monoclonal conjugates also reacted with the type-specific standard antiserum.

and influenza B are circulating concurrently.

In addition to their sharp specificity, other advantages of hybridoma-derived monoclonal antibodies are the facts that they are available in virtually unlimited quantities from frozen stocks of antibody-producing hybrid cells and that the conjugates of immune ascitic fluids can be used at remarkably high dilutions as compared to standard antiserum conjugates, providing a large and consistent supply of the immune reagents.

It must be recognized, however, that the narrow specificity of monoclonal antibodies may be a drawback as well as an advantage. If antigenic drift in the hemagglutinin of an influenza A virus were to involve the determinant against which the monoclonal antibody is directed, the conjugate would no longer be useful for identification. However, this would not lead to an incorrect identification if a type-reactive influenza A conjugate were included in the identification scheme; staining with this conjugate, but not with a strain-specific one, would indicate that the isolate was an influenza A virus no longer covered by the strain-specific reagents, and new reagents to the current strain would have to be prepared.

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