Use of Monoclonal Antibodies for Rapid Detection of Influenza A Virus in Nasopharyngeal Secretions


Two monoclonal antibodies against influenza A virus were assessed for use as diagnostic reagents in an indirect immunofluorescence assay (IFA) of nasopharyngeal secretions. Monoclonal antibody IA-52, directed at an internal antigen, reacted with all influenza A tested. The high stability of this epitope permitted its use in a rapid IFA test, which gave results comparable to those obtained with polyclonal antibodies and viral isolation. The second monoclonal antibody, IA-279 was directed at a surface epitope (hemagglutinin); it reacted with almost all H3 subtype strains. Positive IFA using these monoclonal antibodies permitted rapid preliminary differentiation between the current two major subtypes of influenza A virus (H1N1, H3N2).

A valuable alternative to viral isolation (1), especially for influenza A (2-9), is the rapid detection of viral antigens in nasopharyngeal secretion by means of the immunofluorescence assay (IFA). The early detection of the onset of an epidemic and its comprehensive surveillance reduce the risk of nosocomial influenza infections (10-12). However, owing to difficulties in obtaining specific and sensitive antisera, the use of standard polyclonal antibodies has been limited to only a few laboratories. Monoclonal antibodies offer a number of advantages for diagnosis of respiratory viral diseases in terms of specificity, sensitivity, reproducibility and wide availability. Such antibodies have been used to diagnose respiratory syncytial virus (RSV) (13, 14) and parainfluenza III virus (15) infections and to identify influenza A isolates in cell culture (16). More recently a pool of monoclonal antibodies was used to diagnose influenza A and B (17, 18) by IFA of nasopharyngeal cells.

We report the findings of a multicentre study which evaluated two monoclonal antibodies, one reacting against a group-specific antigen and the other against hemagglutinin (H3).

Materials and Methods

Production of Hybridomas and Characterization of Monoclonal Antibodies. Immunization and preparation of hybrids was according to published procedures (18). Briefly, Balb/c mice were inoculated with influenza A/Texas/1/77 (H3N2) virus grown in allantoic fluids. Spleen cells fused with SP2/O-Agl4 myeloma cells were placed in 96-well plates. About 10 days after fusion the supernatant fluids were tested by indirect immunofluorescence for the presence of antibodies against Chang conjunctiva cells (CCL.20.2 Flow Laboratories, France) infected with influenza A/Texas/1/77 subtype H3N2 or A/USSR/90/77 subtype H1N1. A control was made using uninfected cell culture. The positive hybrids were cloned twice in 1.5 % methyl cellulose, grown further in flasks and tested in pristane-primed mice. The hybridoma supernatant fluids were tested by indirect immunofluorescence for the presence of antibodies against Chang conjunctiva cells (CCL.20.2 Flow Laboratories, France) infected with influenza A/Texas/1/77 subtype H3N2 or A/USSR/90/77 subtype H1N1. A control was made using uninfected cell culture. The positive hybrids were cloned twice in 1.5 % methyl cellulose, grown further in flasks and tested in pristane-primed mice. Later the monoclonal antibodies were tested by indirect IFA on Chang conjunctiva cells infected with different reference strains of H1N1, H2N2, H3N2 human subtypes and their following variants: A/Puerto Rico/8/34 (H2N2), A/USSR/90/77 (H1N1), A/Brazil/1/78 (H2N1), A/Japan/305/57 (H2N2), A/Hong Kong/1/68 (H3N2), A/England/42/72 (H3N2), A/Port Chalmers/1/73 (H3N2), A/Victoria/3/75 (H3N2), A/Texas/1/77 (H3N2), A/Bangkok/1/79 (H3N2), A/Shanghai/31/80 (H3N2), and A/Philippines/2/82 (H3N2). A second control was made using cells infected with the most important viral respiratory agents (influenza B, RSV, parainfluenza 3, 2, 1) and measles. The IFA antibody titer was determined once in fresh supernatant and six months later at +4 °C to determine the stability of the antibodies. Supernatant treated with receptor destroying enzyme (RDE) was evaluated in a micro-inhibition hemagglutination test according to standard procedures (19). The class and subclass of immunoglobulins were identified by immunodiffusion tests with rabbit anti-mouse antibodies (Nordic Immunological Laboratories, UK).

Sampling. Nasopharyngeal secretions were obtained from patients hospitalized in Caen, Dijon, Lyon or Paris in the winter of 1984–1985 during an outbreak of influenza A infection. In addition, samples of 50 healthy controls were taken from the Dijon hospital during a nonepidemic period.
Samples were collected and processed by the method of Gardner and McQuillin (1) with minor modifications. Briefly, after the nasal cavities were washed with 0.5 ml phosphate buffered saline (PBS), nasopharyngeal secretions were collected through the nostrils with a mucous suction pump. The samples were brought to the laboratory within one hour. The cell suspension was then centrifuged at 1500 rpm for 10 min. The supernatant was kept for eventual viral isolation. The cells were washed three times, resuspended in a small volume of PBS, distributed evenly on acetone-resistant microscope slides, which were air-dried and fixed in cold acetone for 10 min.

**Immunofluorescence Assay.** A standard IFA was used. The smears were coated with either 20 μl of commercial broadly reactive bovine anti-influenza A serum diluted 1:5 in PBS (Wellcome Diagnostic, UK) or 20 μl of rabbit anti-influenza A/Bangkok/1/79 (H3N2) (kindly provided by C. Hannoun, Institut Pasteur, France), and 20 μl of undiluted supernatants of each monoclonal antibody (IA-52, IA-279). In some cases supernatants of monoclonal antibodies diluted 1:10 were also tested. The slides were incubated in a humidified chamber at 37 °C for 1 h, washed in PBS and drained. The smears were then coated with either 20 μl of fluorescein-conjugated rabbit anti-bovine immunoglobulin G (Weilcome Diagnostics, UK), goat anti-rabbit immunoglobulin G or sheep anti-mouse immunoglobulin G (Diagnostics Pasteur, France) at the optimum dilution (respectively, 1:10, 1:50 and 1:100). The slides were reincubated for 1 h at 37 °C, washed, drained, and mounted with buffered glycerol (pH 8.0). The slides were examined by two observers using an ultraviolet microscope at magnifications of × 250 and × 400, and the results were compared. The IFA tests were performed on the same slide with other antibodies against influenza B, RSV and parainfluenza 3 virus.

**Viral Isolation.** In some cases supernatants of samples were inoculated in duplicate on primary vervet monkey kidney cells (Flow Laboratories) and incubated at 35 °C on a roller drum. The cultures were examined for hemadsorption to guinea-pig red blood cells after incubation for 8–10 days. A blind passage was routinely performed if hemadsorption was negative. Positive samples were sent to the influenza national center for northern France (Professor C. Hannoun) or southern France (Professor M. Aymard) for confirmation and further strain identification.

**Results**

Two hybridomas were selected. One clone (IA-52) produced a monoclonal antibody (IgG2a) which showed no hemagglutination inhibition when combined with the homologous strain (A/Texas/1/77, H3N2). This monoclonal antibody was directed against an internal antigen-specific group and reacted in IFA against all subtypes and variants tested (H1N1, H2N2 and H3N2). The antibody titers measured using cells infected with A/Texas/1/77 and A/USSR/90/77 were 1:80 in culture medium and 1:30,000 in ascitic fluid. The second clone IA-279 produced a monoclonal antibody (IgG2a) which was directed against hemagglutinin, showed hemagglutination inhibition when combined with the homologous strain and had an antibody titer above 1:40. This monoclonal antibody reacted in IFA against all variants of the subtype H3N2 tested. The IFA titers determined for each strain with A/Texas/1/77 were 1:160 in culture medium and 1:40,000 in ascitic fluid. Non-specific fluorescence was observed neither on uninfected cell cultures nor on the healthy control samples. Cross-reactions were not detected between these two monoclonal antibodies and other viral respiratory agents (influenza B, RSV, parainfluenza virus) or measles. Both monoclonal antibodies were stable.

In the multicentre study 95 nasopharyngeal samples were positive using the undiluted culture medium of monoclonal antibody IA-52. When the supernatant was diluted 1:10, 45 of 46 samples tested were positive. Rough granular, intracytoplasmic inclusions were observed, but non-specific fluorescence was not (Figure 1A). All samples were also positive with the polyclonal antibodies; however, the intensity of fluorescence was weaker in two samples.

**Viral isolation** was attempted in 60 of 95 nasopharyngeal samples (Table 1); 57 samples were positive. Thirty-five of these strains were identified as H3N2 (A/Philippines/2/82 and A/Caen/1/84), and eight as H1N1 (A/Brazil/1/78). All samples found to be negative with polyclonal antibodies and viral isolation...