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Monoclonal Antibodies Against Respiratory Syncytial Virus and Their Use for Rapid Detection of Virus in Nasopharyngeal Secretions

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We developed five monoclonal antibodies against respiratory syncytial virus. Three of these ($23A_3$, $12A_4$, and $18B_2$) were used in an indirect fluorescent antibody test, and the results were compared with those of a similar indirect fluorescent test with commercial anti-respiratory syncytial virus serum. The results obtained with antibody $18B_2$ and commercial anti-respiratory syncytial virus serum were identical, whereas with antibodies $23A_3$ and $12A_4$ the incidence of positive identifications was around 50%.

Respiratory syncytial virus (RSV) is the main cause of acute respiratory infections in young children (7, 9, 16). In recent years, rapid diagnosis by immunofluorescence with the use of standard antibodies has been developed (2, 12, 14). However, owing to difficulties in obtaining specific and sensitive antisera, the use of this method of diagnosis was limited to a few laboratories. Furthermore, the variations among different commercially available sera, and even among batches from the same supplier, make it difficult to obtain reproducible results. To avoid these difficulties, we have produced monoclonal antibodies against RSV and have used them to detect RSV antigens in the nasopharyngeal secretions of children with respiratory infections.

The strain of RSV used for antibody production was isolated during the winter of 1980 from a child at the Trousseau hospital in Paris, grown in cell culture, and stored at −70°C. Immunization and preparation of hybrids were done by published procedures (4, 11). About 12 days after fusion, the supernatant fluid from the wells in which the hybrids were grown were tested by indirect immunofluorescence for the presence of RSV monoclonal antibodies. Fluorescein-labeled anti-mouse antibodies (Institut Pasteur Production, Paris, France) were used at a dilution of 1:100. The test was considered to be positive when the intracytoplasmic fluorescence characteristic of RSV was observed; the controls were negative. The positive hybrids were cloned twice by limiting dilutions onto 96-well plates and injected into pristane-primed mice. The class and subclass identification of the secreted immunoglobulin was done by immunodiffusion tests with rabbit anti-mouse antibodies (Nordic Immunological Laboratories).

Samples of the nasopharyngeal secretions were obtained from children hospitalized at either the Trousseau hospital or the Dijon hospital during the winters of 1982 to 1983 and 1983 to 1984. The samples were treated by the procedure of McQuillin and Gardner (14). Two different people examined the slides by immunofluorescence either on the day of the sampling or after the slides were held at −20°C for one day, and the results were compared.

A standard indirect immunofluorescence method was used (14). The smears were coated with either 20 μl of commercial bovine anti-RSV serum diluted 1:5 in phosphate-buffered saline (Wellcome Diagnostic) or 20 μl of monoclonal ascitic fluid diluted 1:100 in phosphate-buffered saline for monoclonal antibodies $23A_3$, $12A_4$, and $18B_2$ when used alone; 1:200 for each monoclonal antibody when pools of $23A_3$ and $12A_4$, $23A_3$, and $18B_2$, $12A_4$ and $18B_2$ were used; or 1:300 for each monoclonal antibody when a pool of $23A_3$, $12A_4$, and $18B_2$ was used. The slides were incubated in a humidified chamber at 37°C for 1 h, washed three times in phosphate-buffered saline, and drained. The smears were then coated with 20 μl of a 1:10 dilution of the fluorescein-conjugated rabbit anti-bovine immunoglobulin G (IgG; Wellcome Diagnostic) or 20 μl of a 1:100 dilution of the fluorescein-conjugated sheep anti-mouse IgG (Institut Pasteur Production). The slides were reincubated, washed, drained, and mounted with buffered glycerol (pH 8.0). The slides were examined under a UV microscope at magnifications of ×250 and ×400.

Five hybridomas producing monoclonal antibodies against RSV were cloned: hybridomas $12A_4$ and $10C_4$ secreted an IgG2a, and hybridomas $11A_1$, $23A_3$, and $18B_2$ secreted an IgG1. The ascitic fluids obtained were tested by immunofluorescence. The antibody titers in the ascitic fluid, expressed as the reciprocal of the highest dilution giving a specific fluorescence on RSV-infected HeLa cells, were as follows: 1,000 for $11A_1$, $23A_3$, and $10C_4$; 4,000 for $12A_4$; and 32,000 for $18B_2$. Initially, immunofluorescence tests were done on 29 samples simultaneously with the bovine RSV antibodies and with three monoclonal antibodies ($23A_3$, $12A_4$, and $18B_2$), either singly or together (Table 1). Nineteen samples were shown to be positive with the bovine antibodies (Wellcome) and monoclonal antibody $18B_2$. Antibodies $23A_3$ and $12A_4$ gave only nine positive results but were not on the same specimens. Both antibodies gave positive results with five strains; antibody $23A_3$ gave positive results with four others, for which antibody $12A_4$ gave negative results; and antibody $12A_4$ gave positive results with another four strains, for which antibody $23A_3$ gave negative results. We did not see selective reactions of the monoclonal antibodies with viruses from one winter and not from the other. Combining monoclonal antibodies $23A_3$ and $12A_4$ increased the number of positive samples detected (Table 1). Of the 10 samples shown to be negative with bovine antibodies and by viral

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TABLE 1. Results of detection of RSV antigen by immunofluorescence tests with three monoclonal antibodies in comparison with an immunofluorescence test with commercial anti-RSV antibodies and with cell culture (29 specimens)

mIFA[a] test	No. (%) of specimens (n = 19) positive by cell culture and cIFA test[a]
$23A_3$	9 (47.4)
$12A_4$	9 (47.4)
$18B_2$	19 (100)
$23A_3$ and $12A_4$	15 (79)
$23A_3$ and $18B_2$	19 (100)
$12A_4$ and $18B_2$	19 (100)
$23A_3$, $12A_4$, and $18B_2$	19 (100)

[a] mIFA, Immunofluorescence test with monoclonal antibodies; cIFA, immunofluorescence test with commercial anti-RSV antibodies. All specimens (10) negative with the commercial antibodies and cultures were also negative with the three monoclonal antibodies.

isolation, none were found to be positive when tested with our monoclonal antibodies. Immunofluorescence tests were run on 215 samples in parallel with the bovine antibodies (Wellcome) and monoclonal antibodies $18B_2$. Identical results were obtained with these two antibodies (65 positive), although we found a variation in the intensity of fluorescence with some samples. Immunofluorescence tests at different concentrations of $18B_2$ antibodies were done on 10 samples. Two of the samples were positive up to a 1:16,000 antibody dilution, and eight were positive up to a 1:8,000 dilution.

The diagnosis of RSV in children has taken on an increasing importance as a result of the frequency of infection in the pediatric environment (7, 16) and the possibility of treating severe infections with ribavirine (8) or other antiviral agents. Virus isolation usually gives results too late to be useful to clinical physicians. In 1968, McQuillin and Gardner devised an immunofluorescence technique that enabled them to get rapid results comparable (2, 9, 12, 13, 15) or superior (2, 5) to virus isolation in cell culture. However, the specific fluorescence of RSV varies with the antisera used, which can make interpretation difficult. The use of monoclonal antibodies gives clear staining of cytoplasmic inclusions with very little nonspecific fluorescence. The results obtained with $18B_2$ antibody corresponded completely to those obtained with generally used bovine antibody (Wellcome) (1). The staining patterns obtained with this monoclonal antibody, irrespective of the intensity of the fluorescence for a given sample, have always been easily recognizable. The results obtained with $23A_3$ and $12A_4$ antibodies were markedly different, since the incidence of positive specimens identified was only 47.4%. This difference could be due to these antibodies having a lower affinity for their antigenic sites than $18B_2$. The titrations of $23A_3$ and $12A_4$ antibodies in ascitic fluids support this hypothesis. It is, however, also possible that the epitopes detected by the monoclonal antibodies are different, $18B_2$ antibody reacting with an antigen present in most of the strains of human RSV and $23A_3$ and $12A_4$ antibodies reacting with antigens present only in some strains (6). Such antigenic variations between strains of human RSV would be epidemiologically important. Monoclonal antibodies able to differentiate between different isolates of RSV would be one tool for typing this virus.

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