MONOCLONAL ANTIBODIES AGAINST RESPIRATORY SYNCYTIAL VIRUS FOR RAPID DETECTION AND SUBGROUP DETERMINATION

Surang Tantivanich[1], Malinee Chittaganpitch[2], Srisin Khusmith[1] and Sirima Pattamadilok[2]

[1]Department of Microbiology and Immunology, Faculty of Tropical Medicine, Mahidol University, Bangkok 10400; [2]Department of Medical Science, National Institute of Health, Nonthaburi 11000 Thailand

Abstract. Thirty-one hybridomas producing monoclonal antibodies (MAbs) against structural proteins of RSV subgroup A (Long strain) and RSV subgroup B (Japanese wild strain) were produced and seperated into three groups by their reactivities with RSV-A and RSV-B using IFA. Group I was specific to RSV-A, Group II was specific to RSV-B and group III was specific to both subgroups. Characterization of selected two MAbs from each group indicated that three MAbs recognized phosphoprotein (P) and the others recognized fusion protein (F). All of the selected MAbs were IgG_1 and carried kappa light chain. These selected MAbs can be used to detect the presence of RSV from NPAs and classify them into two subgroups. The infection rates of RSV in Thai children are very low and most of them were RSV subgroup A.

INTRODUCTION

Respiratory syncytial virus (RSV) is a major cause of lower-respiratory tract illness in infants and young children worldwide (Pan American Health Organization, 1982). In Thailand, a study by Tantivanich *et al* (1984) indicated that RSV was the major causative agent of acute respiratory tract infection. The appropriate use of antiviral agents to treat RSV infections will be assisted by readily available methods of rapid diagnosis, and the rapid detection of RSV from clinical specimens is also useful in limiting nosocomial spread (Kaul *et al*, 1978; Mintz *et al*, 1979). Several methods have been developed for detection of RSV from clinical specimens, *eg* IFA, PCR, and tissue culture. These tests have been widely used in research work, and the most frequently used test that is performed in the laboratory is IFA. In order to obtain reliable results, an experienced person in needed. In addition, the lack of uniform standardization and precise characterization of commercially available antisera has made it difficult to compare the results obtained with different lots of reagents.

The development of monoclonal antibodies (MAbs) to RSV offers the opportunity for improvement in laboratory handing of the virus; they are preferable to animal anti-RSV sera for use in antigen detection tests.

The purpose of this study was to produce specific MAbs that will be useful for rapid detection of RSV from clinical specimens and to distinguish between RSV subgroups A and B for epidemiological studies.

MATERIALS AND METHODS

Virus strain and preparation of virus materials

RSV subgroup A (Long strain) and RSV subgroup B (Japanese wild strain) were kindly supplied by Sapporo Medical College, Sapparo, Japan. Both strains were propagated in Hep-2 cells and maintained in MEM containing 2% fetal bovine serum. The virus contents were checked by plaque titration.

Production of hybridoma cell lines

Purified RSV subgroup A and B at titers of 10^5 to 10^6 PFU/ml were used for immunization of BALB/C mice. The procedure for immunization of mice and production of hybridoma cell lines followed the methods of Gimenez *et al* (1984) and of Orvell and Grandien (1982).

IFA was used for screening specific antibody producing hybrids, determination of RSV from nasopharyngeal aspirates (NPAs), subgroup determination and confirmation of the NPAs isolated. ELISA was used to characterize MABs by following the method of Engvall and Perlmann (1972).

Specification of MAbs to RSV protein were done by using SDS-PAGE and Western blot analysis according to the method of Dimitrov *et al* (1984) and Towbin *et al* (1979), respectively.

Isotyping of MAbs, class and subclass determination were determined by using Mouse Typer kit (Bio Rad, USA).

Bulk MAbs were prepared by growing the selected hybridoma clone in the intraperitoneal cavity of BALB/C mice. After 14 days, the ascites fluids were collected and purified by centrifugation. Further purification was done by using Protein A-Sepharose 4 B affinity chromatography according to the method of Johnstone and Thorpe (1982) with slight modifications. The protein A-Sepharose CL-4 B (Pharmacia, Sweden) was placed in a 5 ml syringe with glass wool at the bottom end and washed with 0.1 M acetic acid. The column was equilibrated with 0.1 M phosphate buffer pH 8.0, after which the pH of the ascites fluid was adjusted to 8.0 with 1 M Tris HCl pH 9.0 applied with a flow rate of 5 ml/hour. IgG_1, IgG_{2a} and IgG_{2b} were eluted from the column using citrate buffers at pH 6.0, pH 4.5, and pH 3.5, respectively.

Confirmation of MAbs and subgroup determination

Thirty RSV strains isolated from the children in Bangkok during 1988 to 1994 were inoculated into Hep-2 cells. After CPE developed, the infected cells were harvested and fixed on slides for IFA. One selected MAb from each group and reference MAbs (Sapparo Medical College, Sapparo, Japan) were tested with the RSV isolates.

Detection of RSV from NPAs by using MAbs

Nasopharyngeal aspirates (NPAs) were obtained from 2 groups of children. The first group comprised 204 infants admitted to the Children's Hospital with acute respiratory tract infection during June to August 1992. Their ages varied from 1 to 6 months. The second group comprised 10 children admitted to Phramongkutklao Hospital during May to June 1996: their ages varied from 2 months to 3 years. NPA collection was done using a catheter with a specimen trap for aspirating the mucus, which was transported to the laboratory in an ice-box. Each specimen was vigorously vortexed and the mucus disrupted by pipetting before centrifuging at 1,500 rpm for 10 minutes. The supernatants were kept at -70°C until testing. Isolation of RSV from NPAs was done by infecting the specimens into Hep-2 cells maintained in MEM with 2% fetal bovine serum. The specimens that produced CPE were confirmed by IFA, and subgroup determination was also done by IFA using MAbs produced in this study.

Detection of RSV from NPA by DA-ELISA

Monoclonal antibody was diluted in coating buffer to give 5 µg of protein/ml and 100 µl of the diluted monoclonal antibody was added to each well of a maxisorp immuns module 96 well plate (Nunc, Denmark). The plate was incubated at 4°C overnight in a humidified chamber and washed three times with PBS-Tween. One hundred microliters of sonicated NPAs, viral isolates, control cells, and transport medium were added to each well and incubated at 37°C for 1.5 hours in a humidified chamber. After washing with PBS-Tween 3 times, 100 µl of rabbit anti-RSV (Dakopatt, Denmark) diluted 1:4,000 in PBS was added to each well and incubated at 37°C for 1 hour. The plate was then washed with PBS-Tween 5 times. One hundred microliters of goat anti-rabbit peroxidase conjugated (Dakopatt, Denmark) diluted 1:2,000 in PBS was added and incubated for 1 hour at 37°C. After washing five times with PBS-tween, 100 µl of OPD was added to each well, and the reaction stopped with 2N H_2SO_4. The OD was measured by a spectrophotometer at 492 nm. The cut-off value of DA ELISA was determined by using the formula $X + 3\,SX$.

RESULTS

Six MAbs against RSV subgroup A, five MAbs against RSV subgroup B and twenty MAbs specific to both subgroup A and B were produced (Table 1). Two MAbs that gave strong reactions with RSV antigen from each group were selected for further characterization and bulk production. The specificities of these MAbs were assessed by reacting them with other respiratory viruses, *eg* influenza virus group A, influenza virus group B, adenovirus type 2, and measles virus. No cross reaction was found. The specificities of these MAbs to RSV

Table 1

Production of monoclonal antibodies and their specificities.

Monoclone designation	Strain specificity
Group 1	
2A-1, 5A-1, 6A-1, 18A-1, 1A-2 13A-2	Subgroup A
Group 2	
5B-122, 22B-122, 42B-122, 50B-122, 60B-122	Subgroup B
Group 3	
1C-27, 4C-27, 1C-53, 2C-53, 4C-53, 2C-99, 5C-99, 11C-99, 15C-99, 2C-148, 5C-148, 7C-148, 7C-153, 11C-153, 12C-153, 16C-153, 3C-214, 9C-214, 13C-214, 15C-214	Subgroup A and subgroup B

proteins were also assessed using SDS-PAGE and determined by immunoprecipitation on preblotted nitrocellulose membranes. It was found that 3 MAbs (2A-1, 4C-27 and 1C-53) reacted with P protein (36 K), the other MAbs (13A-2, 5B-122, and 42B-122) reacted with F protein (56 K) as demonstrated in Fig 1.

The class and subclass of these MAbs were IgG_1 and they carried kappa type light chain (Table 2).

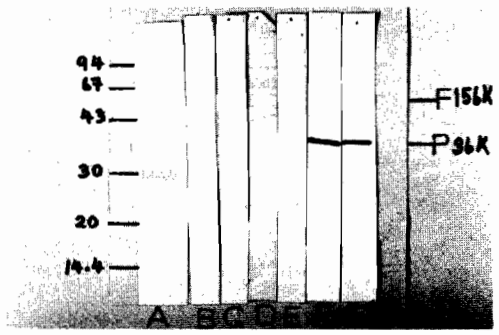

Fig 1–Western blot analysis of monoclonal antibodies against RSV subgroup A and subgroup B antigens.
Lane A = Low molecular weight markers
Lane B = clone 2A-1
Lane C = clone 13A-2
Lane D = clone 5B-122
Lane E = clone 42B-122
Lane F = clone 4C-27
Lane G = clone 1C-53

Subgroup determination of 30 RSV isolated by using these MAbs indicated that 21 isolates were RSV subgroup A and 9 isolates were RSV subgroup B (Table 3). These results were agreed with the results using reference MAbs from Japan.

Four out of 204 NPAs from the Children's Hospital and 3/10 NPAs from Phramongkutklao Hospital gave positive results by tissue culture and IFA. All were RSV subgroup A. The best MAb for performing DA-ELISA is 4C-27 because of its strong reaction with both subgroups. Detection of 44 random NPAs by tissue culture found that 4/44 gave positive results but all of them gave negative results by DA-ELISA.

DISCUSSION

The availability of rapid diagnosis of RSV infections could influence the clinicians' decisions *eg* possible initiation of ribavirin therapy, discontinuation of antibiotics and institution of appropriate infection control measures. Several antigen detection tests, including IFA and EIA, are currently available for rapid diagnosis of RSV. The development of MAbs to RSV provides a more desirable source of antibody because of their specificity and their ample supply.

In the present studies, MAbs to RSV subgroup A and RSV subgroup B were developed. The myeloma cells used to produce MAbs were SP2/0 cells for RSV-A and P_3X_{63} cells for RSV-B. The reasons for using different myeloma cells were the different amounts of specific antibodies produced. These results were similar to previous reports (Gimenez *et al*, 1984; Khusmith *et al*, 1984; Mufson *et al*, 1985; Walsh and Hruska, 1983).

Determination of the specificities of MAbs to specific protein bands found that MAbs to RSV-A did not react with the same protein since one MAb to RSV-A reacted with P protein while another reacted with F protein. All MAbs to RSV-B reacted with F protein and the common group MAbs reacted with P protein. These results were correlated with those in the studies by Mufson *et al* (1985), Norrby *et al* (1986), Johnson and Collins (1988) in that the major antigenic and amino acid sequence differences between two subgroups are found in G-protein (5% antigenically related, 53% amino acid sequence homology) and minor differences are

Table 2

Characteristics of selected monoclonal antibodies against RSV.

Monoclone designation	Isotype	RSV protein specificity	Strain specificity	
			RSV subgroup A	RSV subgroup B
2A-1	IgG_1	phosphoprotein	+	-
13A-2	IgG_1	fusion protein	+	-
5B-122	IgG_1	fusion protein	-	+
42B-122	IgG_1	fusion protein	-	+
4C-27	IgG_1	phosphoprotein	+	+
1C-53	IgG_1	phosphoprotein	+	+

Table 3

Detection of RSV-isolates by IFA using monoclonal antibodies clone 13A-2, clone 5B-122 and clone 4C-27.

Group	RSV-isolates	IFA result		
		clone 13A-2	clone 5B-122	clone 4C-27
I	CD 136/88	+	-	+
(RSV-A)	CD 151/88	+	-	+
	CD 161/88	+	-	+
	R 156/92	+	-	+
	R 172/92	+	-	+
	R 191/92	+	-	+
	R 196/92	+	-	+
	RA 132/92	+	-	+
	CD 56/92	+	-	+
	HC 122/93	+	-	+
	HC 127/93	+	-	+
	HC 128/93	+	-	+
	HC 129/93	+	-	+
	HC 130/93	+	-	+
	HC 133/93	+	-	+
	HC 194/93	+	-	+
	HC 211/93	+	-	+
	Ad 83/93	+	-	+
	CD 4/94	+	-	+
	CD 15/94	+	-	+
	HC 206/94	+	-	+
II	CHU 76/90	-	+	+
(RSV-B)	CHU 154/90	-	+	+
	CHU 156/90	-	+	+
	CHU 176/90	-	+	+
	HC 73/91	-	+	+
	HC 268/90	-	+	+
	HC 306/90	-	+	+
	RA 134/91	-	+	+
	CD 70/92			

found in F-protein (53% antigenically related, 89% amino acid sequence homology).

The reasons for isolation RSV in a small numbers may due to the unusual climate which was extremely dry during the time of collecting specimens since the rainy season came late. Four positive specimens were collected in the last two weeks of the specimen collection period. If the time of collecting specimens were extended, more positive isolates might be expected. Attempts to detect RSV from NPAs by DA-ELISA gave negative results. This may due to the following reasons : 1) A small amount of RSV antigens in the specimens since the CPE showed up very late by tissue culture. 2) The sensitivity of DA-ELISA may not be high enough to detect very small amounts of antigens in NPAs. 3) The selected MAb may react with an epitope which is expressed in small amount within NPAs (Hendy *et al*, 1986). In nasopharyngeal secretions the most abundant RSV polypeptide is the fusion glycoprotein. It is very difficult to develop MAbs against F-protein which is common to all RSV strains. In this study, MAb 4C-27 which is specific to both group A and B was selected because of its high OD value in all RSV isolates but the antigen conformation in NPA specimens might be different from that *in vitro*. Many reports have suggested that using pooled MAbs can increase sensitivity in IFA since they can cover most major antigens in the specimens (Kim *et al*, 1983; Pothier *et al*, 1985; Routledge, 1985). For ELISA, there are many conditions that should be considered before pooling MAbs, *eg* antibodies used for capture and detection should react with distinct and non-overlapping antigenic regions of the polypeptide; their binding capacity to the plastic well plate must be considered.

Even though we failed to use MAbs to detect RSV from NPAs by DA-ELISA, we can still use these MAbs to detect RSV from NPAs and subgroup determination by IFA. The results of this study indicated that most infected Thai children have RSV-subgroup A which is similar to the results of studies in developed countries from the northern hemisphere, and from Uruguay in that subgroup A strains occurred more often than subgroup B strains (Russi *et al*, 1986; Akerlind and Norrby, 1986; Hendry *et al*, 1986; Mufson *et al*, 1986).

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