JOURNAL OF CLINICAL MICROBIOLOGY, June 2002, p. 2220–2223 Vol. 40, No. 6
0095-1137/02/$04.00+0 DOI: 10.1128/JCM.40.6.2220–2223.2002

Evaluation of the Subtype Specificity of Monoclonal Antibodies Raised against H1 and H3 Subtypes of Human Influenza A Virus Hemagglutinins

Eva Varečková,[1]* Nancy Cox,[2] and Alexander Klimov[2]

Institute of Virology, Slovak Academy of Sciences, 842 45 Bratislava, Slovak Republic,[1] and Influenza Branch, National Center for Infectious Diseases, Centers for Disease Control and Prevention, Atlanta, Georgia 30333[2]

Received 11 October 2001/Returned for modification 7 January 2002/Accepted 30 March 2002

Three previously described monoclonal antibodies (MAbs) specific for influenza A(H1) hemagglutinins (HA) revealed high sensitivity (98.2 to 99.1%) and specificity (100%) when tested against 245 strains of different subtypes. One of them was included in the World Health Organization's influenza reagent kit for 2001 to 2002. In contrast, two MAbs raised against human influenza A(H3) HA revealed cross-reactivity with viruses of other subtypes.

The rapid culture assay (RCA) is an effective method for early detection and for typing and subtyping of epidemic viruses directly from clinical specimens within 18 h after infection using specific, well-established monoclonal antibodies (MAbs) (9, 12, 15). Earlier, a panel of MAbs raised against H1 and H3 subtypes of human influenza A virus hemagglutinins (HA) was evaluated in the RCA with a number of human influenza A viruses as well as with other respiratory viruses (9). Three MAbs (12L/5, 13L/6, and 18L/4) reacted exclusively with human viruses of the A(H1N1) subtype. Three other MAbs (IVA1B10, IVG6, and IIF4/D3) reacted with human influenza A viruses of H3, but not H2 or H1, subtypes. These data demonstrated that the MAbs could be useful for subtyping circulating human influenza viruses. However, cases of human infections with influenza A(H5N1) and A(H9N2) viruses (2, 4, 5, 8) indicated a need to verify the specificity of these MAbs with nonhuman influenza viruses. In addition, a new genetic and antigenic group of human influenza A(H1N1) viruses, the A/Beijing/262/95 lineage, appeared in 1995 (1, 13). Viruses of this group have a single amino acid deletion in the HA molecule and are antigenically different from viruses without the deletion (A/Bayern/7/95 lineage). In this study, the MAbs previously shown to be specific to the H1 and H3 subtypes of HA (9) were tested in the RCA with 54 nonhuman influenza A viruses of different subtypes (H1 to H13). The H1-specific MAbs were also tested against different strains from the two currently circulating influenza A(H1N1) lineages, A/Bayern/7/95 and A/Beijing/262/95.

Influenza A viral isolates (a total of 245) of HA subtypes H1 through H13 and influenza B viruses were grown in MDCK cells or in embryonated eggs. Work with pathogenic avian viruses, including A(H5N1) and A(H9N2) human isolates, was conducted under biosafety level 3+ containment. Generation and purification of MAbs 12L/5, 13L/6, 18L/4, IIF4/D3, and IVA1B10 were described earlier (3, 6, 9, 10). In addition, the following MAbs were used as reference antibodies: (i) a mixture of two MAbs specific to influenza A virus nucleoprotein (pool A) (11); (ii) MAb 107L, specific to influenza A nucleoprotein (10); (iii) MAb HA1-71, specific to viruses of the H3 subtype (7, 15); and (iv) a mixture of two MAbs specific to the nucleoprotein and to the HA of influenza B viruses (pool B) (11). The RCA was described previously (9, 15). The following concentrations or dilutions of antibodies were used: 0.35 μg/ml (12L/5 and 13L/6), 0.5 μg/ml (18L/4, IIF4/D3 and IVA1B10), 0.4 μg/ml (107L), 1:400 (HA1-71), 1:1,000 (pool A), and 1:500 (pool B).

Evaluation of MAbs 12L/5, 13L/6, and 18L/4 in the RCA with nonhuman influenza A viruses of different HA subtypes revealed their high specificity. Two MAbs (13L/6 and 18L/4) demonstrated positive reactions with all four tested avian H1 viruses, while MAb 12L/5 reacted with three of them. Nine of 10 swine H1 viruses were positively stained with all three H1-specific MAbs. One swine H1 virus (A/Swine/Wisconsin/1/76) did not react with 12L/5, 13L/6, or 18L/4. These three MAbs did not react with viruses of other subtypes, including several human H5N1 and H9N2 viruses isolated in Hong Kong in 1997 and 1999, respectively (Table 1).

MAbs 12L/5, 13L/6, and 18L/4 had been tested previously for their specificity and sensitivity with 200 human viruses of different subtypes (9). In this study, they were further evaluated with an additional 97 human influenza A(H1N1) viruses isolated during 1994 to 2000 in different countries. Viruses from both cocirculating lineages (A/Bayern/7/95 and A/Beijing/262/95) were examined, including strains similar to a recent antigenic variant from the A/Beijing/262/95 lineage, A/New Caledonia/20/99 (H1N1) (14). Clear perinuclear staining was observed in cells infected with all of these human H1N1 isolates when MAbs 12L/5, 13L/6, and 18L/4 were used (Table 2). No reactivity was detected between these MAbs and 71 recent human A(H3N2) virus isolates, eight historical (1957 to 1968) H2N2 human viruses, and 15 influenza B strains from 1998 to 1999 (data not shown). Thus, the sensitivity of MAbs 12L/5, 13L/6, and 18L/4 was 100% for human viruses and 98.2

* Corresponding author. Mailing address: Institute of Virology, Slovak Academy of Sciences, Dúbravská cesta 9, 842 45 Bratislava, Slovak Republic. Phone: 421-2-59302443. Fax: 421-2-54774284. E-mail: viruevar@nic.savba.sk.

TABLE 1. Reactivity of H1- and H3-specific MAbs with nonhuman influenza A viruses

Virus	Subtype	Reaction with MAb								
		12L	13L	18L	HAI-71	IVA1	IIF4	Pool A	107L	Pool B
A/Swine/Iowa/15/30	H1N1	+	+	+	ND[a]	−	−	ND	+	ND
A/Swine/1976/31	H1N1	+	+	+	ND	−	−	ND	+	ND
A/Swine/Northern Ireland/38	H1N1	+	+	+	ND	−	−	ND	+	ND
A/Swine/Cambridge/39	H1N1	+	+	+	ND	−	−	ND	+	ND
A/Swine/Wisconsin/4/57	H1N1	+	+	+	ND	−	−	ND	+	ND
A/Swine/Illinois/1/63	H1N1	+	+	+	ND	−	−	ND	+	ND
A/Swine/Wisconsin/1/67	H1N1	−	−	−	ND	−	−	ND	+	ND
A/Mayo Clinic/103/74[b]	H1N1	+	+	+	ND	−	−	ND	+	ND
A/NewJersey/08/76[b]	H1N1	+	+	+	ND	−	−	ND	+	ND
A/Swine/Nevada/101/82	H1N1	+	+	+	ND	−	−	ND	+	ND
A/Chicken/Hong Kong/14/76	H1N1	+	+	+	−	−	−	+	+	−
A/Turkey/Kansas/4880/80	H1N1	−	+	+	−	−	−	+	+	−
A/Goose/Hong Kong/8/76	H1N2	+	+	+	−	−	−	+	+	−
A/Duck/Hong Kong/717/79	H1N3	+	+	+	−	−	−	+	+	−
A/Duck/Hong Kong/277/78	H2N2	−	−	−	−	−	−	+	+	−
A/Duck/Hong Kong/77/76	H2N3	−	−	−	−	−	−	+	+	−
A/Duck/Hong Kong/278/78	H2N9	−	−	−	−	−	+	+	+	−
A/Gull/Maryland/19/77	H2N9	−	−	−	−	−	−	+	+	−
A/Mallard/New York/6750/78	H2N2	−	−	−	−	−	+	+	+	−
A/Seal/Massachusetts/3984/92	H3N3	−	−	−	+	+	+	+	+	−
A/Teal/Wisconsin/11/76	H3N6	−	−	−	+	+	+	+	+	−
A/Duck/Ukraine/1/63	H3N8	−	−	−	+	+	+	+	+	−
A/Duck/Wisconsin/8/76	H3N8	−	−	−	+	+	+	+	+	−
A/Equine/Miami/1/63	H3N8	−	−	−	+	+	+	+	+	−
A/Duck/Alberta/630/84	H4N2	−	−	−	−	+	+	+	+	−
A/Seal/Massachusetts/83	H4N5	−	−	−	−	+	+	+	+	−
A/Chicken/Alberta/7394/75	H4N8	−	−	−	−	+	+	+	+	−
A/Hong Kong/156/97[c]	H5N1	−	−	−	−	−	+	+	+	−
A/Hong Kong/491/97[c]	H5N1	−	−	−	−	−	+	+	+	−
A/Hong Kong/483/97[c]	H5N1	−	−	−	−	−	+	+	+	−
A/Turkey/Minnesota/95	H5N2	−	−	−	−	−	+	+	+	−
A/Duck/Singapore/97	H5N3	−	−	−	−	−	+	+	+	−
A/Duck/Wisconsin/4/74	H5N3	−	−	−	−	−	+	+	+	−
A/Duck/Potsdam/1402/86	H5N6	−	−	−	−	−	+	+	+	−
A/Bird/Wisconsin/86	H6N1	−	−	−	−	−	−	+	+	−
A/Mallard/Wisconsin/116/76	H6N5	−	−	−	−	−	−	+	+	−
A/Shearwater/Australia/1/73	H6N5	−	−	−	−	−	+	+	+	−
A/Equine/Prague/1/56	H7N7	−	−	−	−	−	−	+	+	−
A/Seal/Massachusetts/80	H7N7	−	−	−	−	−	−	+	+	−
A/Redknot/Delaware/254/94	H8N2	−	−	−	−	−	+	+	+	−
A/Turkey/Ontario/6118/68	H8N4	−	−	−	−	−	+	+	+	−
A/Mallard/Wisconsin/426/79	H8N4	−	−	−	−	−	+	+	+	−
A/Quail/Hong Kong/G1/97	H9N2	−	−	−	−	−	−	+	+	−
A/Swine/Hong Kong/10/98	H9N2	−	−	−	−	−	−	+	+	−
A/Turkey/Wisconsin/66	H9N2	−	−	−	−	−	−	+	+	−
A/Chicken/Hong Kong/G9/97	H9N2	−	−	−	−	−	−	+	+	−
A/Hong Kong/1073/99[c]	H9N2	−	−	−	−	−	−	+	+	−
A/Quail/Italy/544/66	H10N8	−	−	−	−	−	−	+	+	−
A/Duck/England/56	H11N6	−	−	−	−	−	−	+	+	−
A/Duck/Wisconsin/26/80	H11N9	−	−	−	−	+	−	+	+	−
A/Mallard/Alberta/561/83	H12N1	−	−	−	−	−	−	+	+	−
A/Whale/ME/328/84	H13N2	−	−	−	−	+	−	+	+	−
A/Gull/Massachusetts/50/80	H13N6	−	−	−	−	+	+/−	+	+	−
A/Gull/Maryland/1824/78	H13N9	−	−	−	−	−	−	+	+	−

[a] ND, not done.
[b] Swine virus isolated from humans.
[c] Avian-like virus isolated from humans.

to 99.1% when nonhuman viruses also were included (with 100% specificity for each of these MAbs).

Two MAbs, IIF4/D3 and IVA1B10, raised against A/Dunedin/4/73 (H3N2) virus (6) were also tested for cross-reactivity with 54 viral isolates of various subtypes. Positive cytoplasmic staining with both MAbs was detected in cells infected with all five nonhuman H3 viruses. Additionally, MAb IIF4/D3 demonstrated positive reactions with all tested viruses of the H4, H5, and H8 subtypes as well as with two H2 viruses, one H6 strain, and one H13 isolate. No reaction was observed between this MAb and H1, H7, H9, H10, H11, or H12 viruses. MAb IVA1B10 cross-reacted with all tested H3 and H4 strains, one H11 virus, and two H13 isolates (Table 1).

Thus, 100% specificity of the H1-specific MAbs 12L/5,

TABLE 2. Human influenza A(H1N1) virus strains tested with H1-specific MAbs

Strain	Strain
A/Hong Kong/59/94	A/Caen/1273/98[a]
A/California/02/95	A/Montevideo/657/98
A/Wisconsin/18/95	A/Gunma/1708/98[a]
A/New Jersey/20/95	A/Ibaraki/39/98[a]
A/Arizona/27/95	A/Bangkok/381/98[a]
A/Netherlands/482/95	A/Hong Kong/4337/98
A/Beijing/262/95[a]	A/Florida/12/98
A/CNIC/48/96	A/New York/62/98
A/Nanchang/25/96	A/Virginia/23/98
A/Hong Kong/01/96	A/Mongolia/11/98
A/Hong Kong/410/96[a]	A/Mongolia/12/98
A/Singapore/15/96[a]	A/Ishikawa/42/98[a]
A/Brazil/92/96	A/Ishikawa/43/98[a]
A/Brazil/135/96	A/Kanagawa/92/98[a]
A/Brazil/140/96	A/Thailand/4016/98[a]
A/Brazil/139/96	A/Ningxia/3/98[a]
A/Brazil/232/96	A/Brazil/17/98
A/Brazil/123/97	A/Nanchang/16/98[a]
A/Brazil/124/97	A/Nanchang/104/98[a]
A/Brazil/137/97	A/Nanchang/110/98[a]
A/Brazil/143/97	A/Nanchang/115/98[a]
A/Brazil/186/97	A/Nanchang/119/98[a]
A/Brazil/199/97	A/Nanchang/102/98[a]
A/Bulgaria/107/97	A/Sichuan/4/98
A/Durbin/92/97	A/California/07/99
A/Beijing/206/97[a]	A/Hong Kong/81/99[a]
A/Fujian/222/97[a]	A/Peru/1250/99[a]
A/Guangzhou/105/97[a]	A/Pusan/124/99[a]
A/Nanchang/16/97[a]	A/Taiwan/1382/99[a]
A/Nanchang/19/97[a]	A/Japan/255/99[a]
A/Shenzhen/213/97[a]	A/Florida/05/99
A/Shenzhen/240/97[a]	A/Florida/06/99
A/Wuhan/105/97[a]	A/Peru/1261/99[a]
A/Wuhan/236/97[a]	A/Samara/213/99
A/Wuhan/244/97[a]	A/Whashington/11/99
A/Shanghai/02/97[a]	A/North Carolina/3/99
A/Hong Kong/317/97[a]	A/New Caledonia/20/99[a]
A/Hong Kong/345/97[a]	A/Hong Kong/249/2000[a]
A/Hong Kong/470/97[a]	A/Hong Kong/1243/2000[a]
A/Singapore/10/97[a]	A/Hong Kong/1252/2000[a]
A/Singapore/12/97[a]	A/Hong Kong/1289/2000[a]
A/New York/08/97	A/Hong Kong/1292/2000[a]
A/Russia/268/97	A/Kaliningrad/5/2000[a]
A/Mongolia/742/97[a]	A/Genova/130/2000[a]
A/Mongolia/836/97	A/Lisbon/5/2000[a]
A/Brazil/05/97	A/Barcelona/382/2000[a]
A/Wellington/08/97	A/Madrid/1013/2000[a]
A/Johannesburg/159/97[a]	A/Denmark/39/2000
A/Canada/06/97	

[a] Strain, belonging to the A/Beijing/262/95 (H1N1) genetic/antigenic lineage.

13L/6, and 18L/4 was confirmed in this study. These MAbs demonstrated positive reactions with most tested H1 viruses of nonhuman origin (98.2 to 99.1% sensitivity). Importantly, MAbs 12L/5, 13L/6, and 18L/4 demonstrated 100% sensitivity in detection of viruses from both currently circulating genetic lineages of influenza A(H1N1) viruses, A/Bayern/7/95 and A/Beijing/262/95, including A/New Caledonia/20/99-like viruses (14). Therefore, MAbs 12L/5, 13L/6, and 18L//4 are reliable and sensitive reagents for rapid diagnosis of human influenza A viruses of the H1 subtype.

Antibodies IIF4/D3 and IVA1B10, specific to HA2 and HA1 glycopolypeptides of the H3 HA, respectively (6, 9), were identified earlier as good probes for subtyping human H3 viruses (9). In this study we found that they reacted with nonhuman viruses of different subtypes. Their cross-reactivity with several subtypes of nonhuman isolates should be taken into account when human infection with animal (avian) viruses is suspected.

An RCA that permits type- and subtype-specific detection of influenza viruses in clinical specimens was previously described (15). However, MAb HA2-76, which was used for subtyping influenza A(H1N1) viruses, could differentiate only between H3 and non-H3 (e.g., H1) human strains. This MAb was shown to cross-react with several other subtypes of influenza A viruses, including H9 (7). Replacement of MAb HA2-76 with one of the H1-specific MAbs described here will dramatically improve the specificity of the assay (15) for differentiating between H1 and H3 viruses. Recently, we included MAb 18L/4 in the 2001-2002 World Health Organization (WHO) influenza reagent kits produced at the Centers for Disease Control and Prevention, Atlanta, Ga. Before 2001, these kits included only two MAb pools, one specific for type A (pool A) and one specific for type B (pool B) viruses (11). These pools are used by WHO collaborating laboratories for typing influenza viruses in direct immunofluorescent assays or in the RCA. Adding MAb 18L/4, along with the previously described H3-specific MAb HA1-71 (7), to the WHO kits will improve global surveillance for influenza and increase the ability to differentiate new, potentially pandemic influenza virus strains. Pool A and pool B MAbs allow laboratories to type human influenza A and B viruses, while MAbs 18L/4 and HA1-71 allow laboratories to distinguish between human H1 and H3 isolates. Viral isolates positive in reactions with pool A MAbs but negative in reactions with 18L/4 or HA1-71 must be further tested to determine if influenza A viruses of other subtypes are present. Information received from several WHO collaborating laboratories has confirmed that MAb 18L/4 reacted well with recent (2001 to 2002) H1 viruses.

This work was supported, in part, by the Slovak Academy of Sciences (grants 2/1103/21 and 2/7046/20).

We thank Elena Uličná (Institute of Virology, Bratislava, Slovak Republic) for production of ascitic fluids, and we thank Henrietta Hall, Lynn Cooper (Influenza Branch, Centers for Disease Control and Prevention) and Yipu Lin (Department of Virology, National Institute for Medical Research, London, United Kingdom) for their help in selecting influenza viruses used in this study. We also thank Alan Hay (Department of Virology, National Institute for Medical Research, London, United Kingdom) for helpful discussions and Amynah Janmohamed for help in preparing the manuscript.

REFERENCES

1. **Cox, N. J., and H. L. Regnery.** 1996. Global influenza surveillance: tracking a moving target in a rapidly changing world, p. 591–598. *In* L. E. Brown, A. W. Hampson, and R. G. Webster (ed.), Options for the control of influenza III. Elsevier Science B.V., Amsterdam, The Netherlands
2. **De Jong, J. C., E. C. J. Claas, A. D. M. E. Osterhaus, R. G. Webster, and W. L. Lim.** 1997. A pandemic warning? Nature **389:**554.
3. **Ey, P. L., S. J. Prowse, and C. R. Jenkin.** 1978. Isolation of pure IgG1, IgG2a and IgG2b immunoglobulins from mouse serum using Protein A-Sepharose. Biochemistry **15:**429–436.
4. **Lin, Y. P., M. Shaw, V. Gregory, K. Cameron, W. Lim, A. Klimov, K. Subbarao, Y. Guan, S. Krauss, K. Shortridge, R. Webster, N. Cox, and A. Hay.** 2000. Avian-to-human transmission of H9N2 influenza virus: relationship between H9N2 and H5N1 human isolates. Proc. Natl. Acad. Sci. USA **97:**9654–9658.
5. **Peiris, M., K. Y. Yuen, C. W. Leung, K. H. Chan, P. L. Ip, R. W. Lai, W. K. Orr, and K. F. Shortridge.** 1999. Human infection with influenza H9N2. Lancet **354:**916–917.
6. **Russ, G., K. Poláková, F. Kostolanský, B. Styk, and M. Vančíková.** 1987. Monoclonal antibodies to glycopolypeptides HA1 and HA2 of influenza virus hemagglutinin. Acta Virol. **31:**374–386.

7. **Sanchéz-Fauquier, A., N. Villanueva, and J. A. Melero.** 1987. Isolation of cross-reactive, subtype-specific monoclonal antibodies against influenza virus HA1 and HA2 hemagglutinin subunits. Arch. Virol. **97:**251–265.
8. **Subbarao, K., A. Klimov, J. Katz, H. Regnery, W. Lim, H. Hall, M., Perdue, D. Swayne, C. Bender, J. Huang, M. Hemphill, T. Row, M. Shaw, X. Xu, K. Fukuda, and N. J. Cox.** 1998. Characterization of an avian influenza A (H5N1) virus isolated from a child with a fatal respiratory illness. Science **279:**393–396.
9. **Tkáčová, M., E. Varečková, I. C. Baker, J. M. Love, and T. Ziegler.** 1997. Evaluation of monoclonal antibodies for subtyping of currently circulating human type A influenza viruses. J. Clin. Microbiol. **35:**1196–1198.
10. **Varečková, E., T. Betáková, V. Mucha, L. Soláriková, F. Kostolanský, M. Waris, and G. Russ.** 1995. Preparation of monoclonal antibodies for the diagnosis of influenza A infection using different immunization protocols. J. Immunol. Methods **180:**108–116.
11. **Walls, H. H., M. W. Harmon, J. J. Slagle, C. Stocksdale, and A. P. Kendal.** 1986. Characterization and evaluation of monoclonal antibodies developed for typing influenza A and B viruses. J. Clin. Microbiol. **23:**240–245.
12. **Waris, M., T. Ziegler, M. Kivivirta, and O. Ruuskanen.** 1990. Rapid detection of respiratory syncytial virus and influenza A virus in cell cultures by immunoperoxidase staining with monoclonal antibodies. J. Clin. Microbiol. **28:**1159–1162.
13. **Weekly Epidemiological Record.** 1998. Recommended composition of influenza virus vaccines for use in the 1998–1999 season. Wkly. Epidemiol. Rec. **73:**56–63.
14. **Weekly Epidemiological Record.** 1999. Recommended composition of influenza virus vaccines for use in the 1999–2000 season. Wkly. Epidemiol. Rec. **74:**57–61.
15. **Ziegler, T., H. Hall, A. Sanchéz-Fauquier, W. C. Gamble, and N. J. Cox.** 1995. Type- and subtype-specific detection of influenza viruses in clinical specimens by rapid culture assay. J. Clin. Microbiol. **33:**318–321.