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Letter to the Editor

Simultaneous detection and typing of human metapneumovirus strains in nasopharyngeal secretions and cell cultures by monoclonal antibodies

Dear Editor,

Recently, human metapneumovirus (hMPV) has been identified as a major human viral pathogen and reported to be the etiologic agent of upper and lower respiratory tract infections in infants and young children as well as the elderly and the immunocompromised host (Boivin et al., 2002; Bastien et al., 2003; Falsey et al., 2003; Maggi et al., 2003). Virus has been mostly identified in clinical respiratory samples by reverse-transcription PCR (RT-PCR), but animal immune sera have also been used for hMPV identification both in nasopharyngeal aspirates (NPA) and cell cultures (Van den Hoogen et al., 2001; Percivalle et al., 2005).

More recently, immunological identification of hMPV strains was achieved by direct fluorescent antibody (DFA) staining of cells from NPA samples as well as from inoculated cell cultures using monoclonal antibodies (MAbs) (Ebihara et al., 2005; Percivalle et al., 2005; Landry et al., 2005). Although sensitivity and negative predictive value of MAbs were somewhat lower than those achieved by RT-PCR, rapidity of turnaround time and simplicity of test performance appeared as diagnostic parameters favouring the immunological approach (Percivalle et al., 2005).

All hMPV strains recovered till now in different countries of the five continents have been classified into two major clusters, referred to as types A and B, on the basis of sequencing and phylogenetic analysis of genes L, N, F, or P (Boivin et al., 2002, 2004; Van den Hoogen et al., 2001, 2004).

In the present study, type-specific monoclonal antibodies (MAbs) raised against type A and type B hMPV strains were developed, using virus strains recovered and propagated in LLC-MK2 cell cultures. These MAbs were shown to type all strains previously characterized by sequencing and phylogenetic analysis.

Our prototype A and B hMPV strains (I-PV 03/01 6621 and I-PV 03/04 4702, respectively) propagated in LLC-MK2 cell cultures (Gerna et al., 2005) were concentrated by ultracentrifugation and then inoculated into BALB/C mice according to a reported protocol (Percivalle et al., 2005). Following fusion of mouse spleen cell suspensions with Sp2/0Ag14 myeloma cells, hybridomas were tested for specific reactivity with hMPV by enzyme-linked immunosorbent assay and the indirect fluorescent antibody (IFA) assay. Following cloning and subcloning, MAbs previously selected for specific reactivity with hMPV were tested for type specificity by IFA using our type A and B prototype strains. Specific reactivity with viral proteins was tested by Skiadopoulos at NIH, NIAID (Bethesda, MD) by Western blot of sucrose-purified reference hMPV strains CAN83 (type A) and CAN75 (type B) which were isolated in Canada and represent known prototypes of each of the two major genetic lineages (Peret et al., 2002). In addition, MAbs were tested by IFA on LLC-MK2 cells, which were infected with recombinant human parainfluenza virus type 1 expressing the hMPV fusion (F), small hydrophobic (SH), and the attachment glycoprotein (G) of both Canadian prototypes (Newman et al., 2002; Skiadopoulos et al., 2004).

Two MAbs, including clones F4A1 (IgG1) and CB7F3 (IgG1), each reactive by both DFA and ELISA assays with either type A or type B hMPV strains, respectively, were selected and tested for cross-reactivities with conventional respiratory viruses (influenza viruses A and B, parainfluenza virus types 1–4, human respiratory syncytial virus, human adenovirus, human coronaviruses 229, OC43 and NL63, and rhinoviruses). No cross-reactivity with any of known respiratory viruses was detected for either one of the two selected MAbs. Both type-specific MAbs were found to react with the F protein of the homologous virus type by both IFA and Western blot.

A total of 67 NPA samples were tested by DFA using type-specific MAbs. On the whole, 24 hMPV strains were typed by MAbs on duplicate NPA slides (100% sensitivity). As a result, 16 strains were found to belong to type A, and 8 to type B (Table 1). These results exactly matched those obtained by sequencing and phylogenetic analysis (Gerna et al., 2005). In the meantime, type-specific MAbs were used to test 18 NPA samples positive for different respiratory viruses as reported in Table 1. No cross-reactivity with any of the other respiratory viruses tested was found (100% specificity). In addition, hMPV type-specific MAbs were tested against respiratory cells from 25 NPA samples negative for respiratory viruses. No non-specific reactivity with uninfected respiratory cells was detected (Table 1).

Abbreviations: DFA, direct fluorescent antibody staining; hMPV, human metapneumovirus; MAbs, monoclonal antibodies; NPA, nasopharyngeal aspirate; RT-PCR, reverse-transcription polymerase chain reaction

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Table 1
hMPV typing of 24 hMPV-positive NPA samples by DFA using type-specific MAbs compared to typing by phylogenetic analysis

Respiratory virus	No. NPAs tested	Typing by hMPV MAbs	
		Type A	Type B
hMPV, type A[a]	16	16	0
hMPV, type B[a]	8	0	8
Influenzavirus A	2	0	0
Influenzavirus B	2	0	0
Parainfluenza virus 1–3	3	0	0
Respiratory syncytial virus	3	0	0
Adenovirus	2	0	0
Human coronaviruses	3	0	0
Rhinoviruses	3	0	0
None (cells from NPA)	25	0	0

In addition, 18 NPAs positive for different respiratory viruses and 25 NPA samples negative for respiratory viruses were tested as controls.
[a] As typed by sequencing and phylogenetic analysis.

Furthermore, some NPA samples positive for hMPV were tested for typing following isolation in LLC-MK2 cell cultures. While 12/12 (100%) of samples inoculated as fresh NPAs were recovered in cell cultures and typed, only 8/25 (32%) samples thawed once or twice, could be typed. Thus, both hMPV recovery and typing are optimally achieved by inoculating fresh samples onto cell cultures.

Morphological patterns of the two type-specific MAbs in cell cultures infected with reference strains are reported in Fig. 1. In addition, IFA patterns observed in respiratory tract cells from hMPV-infected NPA samples, as well as in LLC-MK2 cell cultures following hMPV isolation, are reported for both type A and type B hMPV strains in Fig. 2A–D and E–H, respectively. The staining intensity ranged from 1+ to 4+ in different cells, while the staining pattern was similar to the granular pattern of one of three MAbs included in the pool for hMPV detection (Percivalle et al., 2005).

In the present study, we have succeeded in developing type-specific MAbs anti-hMPV, which have been shown to be able to classify all hMPV strains tested into types A or B, exactly matching results given by sequencing and phylogenetic analysis. Since the two hMPV types have been found to circulate at a much different rate in different years (Gerna et al., 2005), hMPV typing may be useful for epidemiological purposes. Typing by MAbs is highly preferable over typing by phylogenetic analysis in terms of practicality, rapidity and cost-effect benefits. Given the 100% sensitivity and specificity of type-specific MAbs with respect to hMPV detection by the MAb pool (Percivalle et al., 2005), detection and typing of new hMPV strains by MAbs

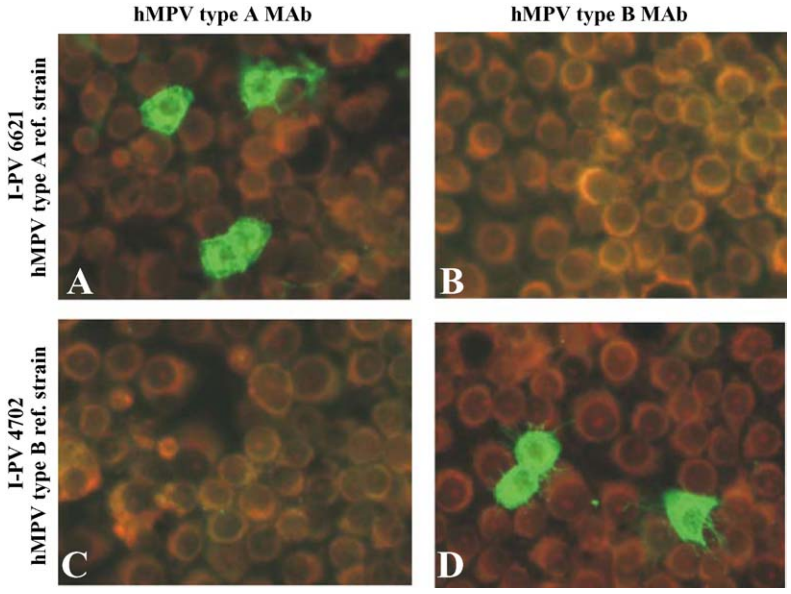

Fig. 1. Typing of reference hMPV strains in LLC-MK2 cell cultures by type-specific MAbs and indirect immunofluorescence (IFA) 24 h p.i. (A and B) Type A (I-PV 03/01 6621); (C and D) type B (I-PV 03/04 4702) reference strains.

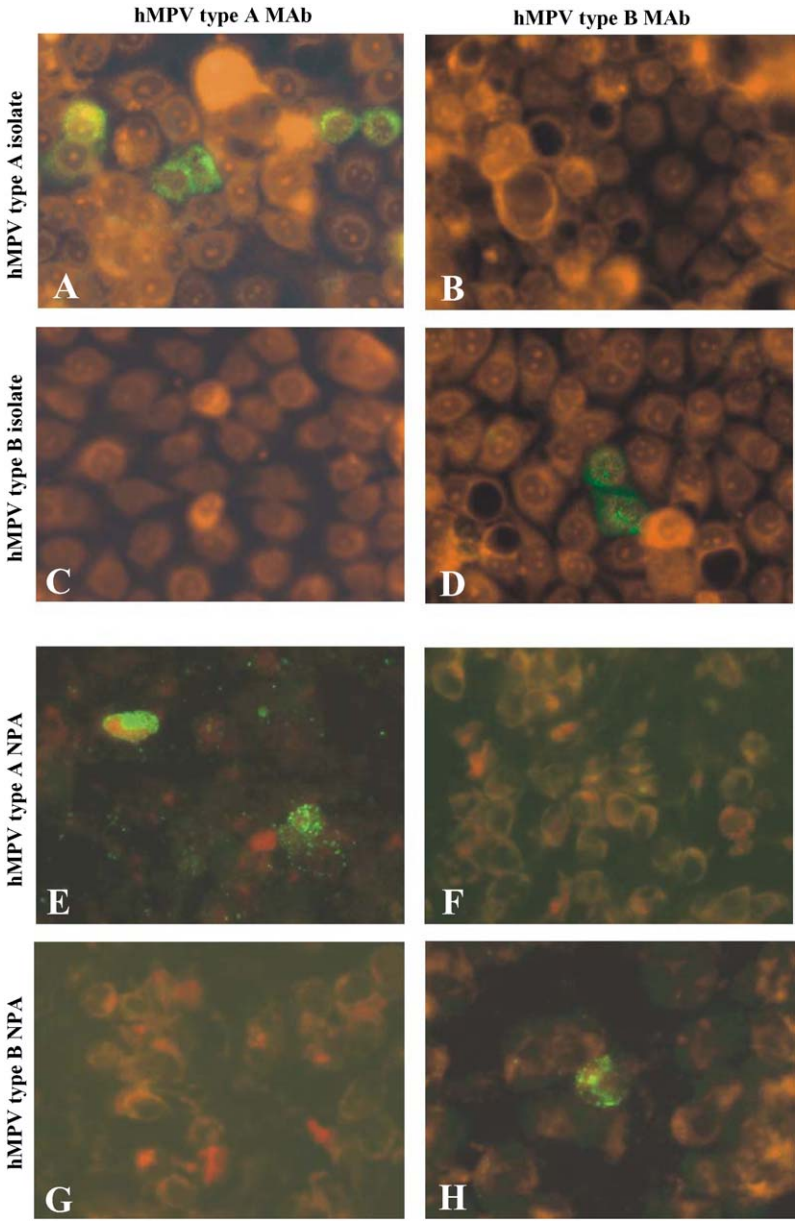

Fig. 2. (A–D) Typing of two hMPV isolates recovered in LLC-MK2 cell cultures 48 h p.i. by IFA and MAbs. (A and B) Type A isolate; (C and D) type B isolate. (E–H) Typing of hMPV strains by IFA and type-specific MAbs on respiratory cells from NPA. (E and F) type A hMPV strain; (G and H) type B hMPV strain.

may be performed simultaneously in viral diagnostic laboratories.

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