Types of *Mycoplasma pneumoniae* in Greece

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ABSTRACT: Throat swab specimens were obtained during 2003 from Greek hospitalized children with acute respiratory tract infection. In order to type *M. pneumoniae* strains a partial region of P1 gene was amplified directly from the clinical specimens and sequence variations among the strains were investigated. It was found that predominant was *M. pneumoniae* type 1.

Key Words: Mycoplasma pneumoniae, Pneumonia, Greece, Genotypes, Molecular epidemiology.

INTRODUCTION

Mycoplasma pneumoniae is a common etiological agent of respiratory tract infections in humans, mainly primary-school children. Epidemic outbreaks of *M. pneumoniae* occur usually at 4 to 7 years intervals. The P1 transmembrane protein of *M. pneumoniae* mediates the attachment of the pathogen to the host cells and elicits a strong humoral immune response during infection in patients[1,2,3]. P1 cytadhesin is a 169-kDa protein encoded by a gene of approx. 5,000 bp, which contains a copy of each repetitive regions DNA elements RepMP2/3 and RepMP4[4]. Su et al.,[5] first classified *M. pneumoniae* strains into two groups on the basis of differences in the P1 gene, and speculated that sequence divergence of the two groups may influence the tropism and virulence of the strains prompting for further comparative studies. Analysis of P1 genes of *M. pneumoniae* isolates by Southern blotting[6], DNA fingerprinting[7], and restriction fragment length polymorphism (RFLP)[8] techniques revealed also two types. On the basis of variation within the P1 gene, Dorigo-Zetsma et al.[9] found eight subtypes in the two groups, while Cousin-Allery et al.[10] using RFLP, RAPD and PFGE found two closely related subgroups into group 2. Recombinant strains have been reported arising by exchanging segments of different genes[11,12]. Recently Dumke et al.[13] using extended genome sequencing and expression profiles concluded that *M. pneumoniae* is a highly uniform species with P1 being the major target for epidemiological screening and that most of the isolates could be assigned to one of the two groups 1 and 2. So far, no relation between types and severity of the disease has been reported.

In Greece, *M. pneumoniae* accounts for approximately 11%[14] of atypical pneumonia cases in children. Laboratory diagnosis is based mainly on serology, while culture of these fastidiously growing bacteria is usually not applied; for this reason no stocked *M. pneumoniae* strains from former years were available. A study on comparison among different diagnostic methods (culture, EIA-antigen detection, PCR, ELISA and western-blot for detection of specific IgG, IgM and IgA antibodies against *M. pneumoniae*) showed that the combination of PCR and IgM-capture ELISA is highly sensitive for rapid diagnosis of acute phase *M. pneumonia* infection in children[14]. Aim of the present study was to type *M. pneumoniae* strains using as starting material throat swab specimens from hospi-

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Table 1. Epidemiological and clinical data of the patients.

No	Date of specimen collection	Age (y)	Gender	Disease	Fever (°C)	WBC X10^9/L	*M. pneumoniae* type
7	June 2003	12	Female	pneumonia	39.0	5,700	Type 1
48	June 2003	8.5	Male	pneumonia	39.5	14,800	Type 1
34	July 2003	10	Male	pneumonia	39.3	12,500	Type 1
51	August 2003	2.5	Male	pneumonia	38.3	14,400	Type 2
56	September 2003	6.5	Female	pneumonia	39.4	10,100	Type 1
64	September 2003	3	Female	pharyngitis	40.5	13,300	Type 1
83	October 2003	9	Male	pneumonia	40	7,600	Type 1

talized children with respiratory tract infection and to gain information on the types of *M. pneumoniae* strains circulating in Greece in 2003.

MATERIALS AND METHODS

During May 2003 to April 2004 throat swab specimens were obtained from 210 children (94 female - 116 male) with mean age 4.4 years (range 2 months to 14 years) who were hospitalized due to acute respiratory tract infection in Thessaloniki, Northern Greece.

Initially a diagnostic PCR was performed, which amplifies a 209-bp region of the P1 adhesin gene[15]. On randomly selected PCR-positive samples a type-specific PCR was applied using primers P1-40 and MPAW1, which amplify a 1110-bp region of the P1 adhesin gene[16]. DNA was extracted directly from throat swab specimens using the Qiagen DNA extraction kit (Qiagen GmbH Hilden, Germany). Sequencing of the PCR products was performed using the ThermoSequenase Cy5.5 Dye Terminator Cycle Sequencing kit (Amersham Biosciences UK Limited) in an OpenGene™ System (Visisble Genetics, Toronto, Ca).

RESULTS

Twenty-four of 210 specimens (11.4%) were found positive using the diagnostic PCR. In fourteen of the positive specimens the type-specific PCR was applied; in seven of them an amplicon of 1110 bp was obtained and sequenced. BLAST analysis shown that six sequences presented 100% similarity with P1 type 1 reference strain M129 (ATCC 29342), while one was identical to P1 type 2 reference strain MAC (ATCC 15492). Epidemiological characteristics and clinical signs and symptoms were similar among all seven patients (Table 1).

DISCUSSION

In the present study it was found that *M. pneumoniae* DNA was detected in 11.4% of hospitalized children with acute respiratory tract infection. Both diagnostic and typing PCRs were performed with DNA extracted directly from throat swab specimens from the patients. Positive results from the typing PCR were taken from half (7/14) of the samples which were positive in the diagnostic PCR. The lower success rate was due to the fact that the size of the typing PCR product was much higher than that of the diagnostic PCR (1110 bp versus 209 bp). It was found that the majority of the *M. pneumoniae* strains belonged to type 1.

On the basis of genotyping most of *M. pneumoniae* strains can be divided into two major types, 1 and 2. *M. pneumoniae* type predominance differs among countries, and even among year of isolation, and in addition, a shift from one subtype to another is observed unevenly. For example, retrospective studies on strains isolated in France and Denmark showed that most of the Danish strains of 1962-86 belonged to type 1, almost all Danish and French strains of 1987-88 belonged to type 2, while during 1991-93 almost all strains (including German and Belgian ones) belonged to type 1 (10). Predominant *M. pneumoniae* type in Germany during the time period 1989 to 1996 was type 1 and in 1997-98 was type 2, while the variant DR was detected only in 1991 and 1995[17]. In Japan, predominance of type 2 was observed in strains isolated in 1979-80 and in 1994-95, while the period in

between (1985-1991) there was predominance of type 1[8]. In Australia type 2 was more commonly implicated in pneumonia cases among children in 1998-99[16]. It is obvious that the distribution of *M. pneumoniae* strains is not homogenous in different regions of the world, and it was suggested that immunoresponse to epitopes that differ in the two groups of P1 proteins influence the relative dominance of one or the other type[8]. Experiments in an animal model showed that pre-infection of the host with *M. pneumoniae* strains of a certain type causes a type-specific immunity with strong influence on the type of the surviving bacteria[13].

Although strains from other years were not available, the present study provided preliminary data on the types of *M. pneumoniae* strains in Greece, where it can be assumed that *M. pneumoniae* type 1 was predominant in 2003. Future studies on strains collected in different countries and time periods will give a better insight into molecular epidemiology of *M. pneumoniae*, and will help to find out any probable relation between types and pathogenicity to humans, and to define the factors implicating in type shifts.

Acknowledgment

Dr. M. Almasri was supported by a grant from the State Scholarships Foundation (IKY), Greece.

Τύποι ελληνικών στελεχών Μυκοπλάσματος της πνευμονίας

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ΠΕΡΙΛΗΨΗ: Κατά τη διάρκεια του 2003 συλλέχθηκαν φαρυγγικά επιχρίσματα από παιδιά που νοσηλεύτηκαν με οξεία λοίμωξη αναπνευστικού συστήματος. Σκοπός της μελέτης ήταν να ανιχνευτεί και να τυποποιηθεί το Μυκόπλασμα της πνευμονίας κατ' ευθείαν από το κλινικό δείγμα, χωρίς καλλιέργεια του βακτηρίου. Για την τυποποίηση του Μ. της πνευμονίας εφαρμόστηκε μία PCR που ενισχύει μία περιοχή του γονιδίου P1, το οποίο ευθύνεται για την προσκόλληση των βακτηρίων στα κύτταρα του ξενιστή. Ακολούθησε αλληλούχιση νουκλεοτιδίων και μελετήθηκαν οι γενετικές διαφορές μεταξύ των στελεχών. Μυκόπλασμα της πνευμονίας ανιχνεύτηκε σε ποσοστό 11,4% των δειγμάτων, με επικρατέστερο των τύπο 1.

Λέξεις Κλειδιά: Μυκόπλασμα πνευμονίας, Πνευμονία, Ελλάδα, Γονότυποι, Μοριακή επιδημιολογία.

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