Case Report

Recurrent infection with genetically identical pneumococcal isolates in a patient with interleukin-1 receptor-associated kinase-4 deficiency

Judit Szabó,¹ Orsolya Dobay,² Melinda Erdős,³ Ágnes Borbély,¹ Ferenc Rozgonyi² and László Maródi³

Correspondence

László Maródi

lmarodi@jaguar.dote.hu

¹Department of Medical Microbiology, Medical and Health Science Centre, University of Debrecen, Debrecen, Hungary

²Institute of Medical Microbiology, Faculty of Medicine, Semmelweis University, Budapest, Hungary

³Department of Infectious and Paediatric Immunology, Medical and Health Science Centre, University of Debrecen, Nagyterdei Krt 98, Debrecen H-4032, Hungary

Interleukin-1 receptor-associated kinase (IRAK)-4 deficiency is a rare primary immunodeficiency disorder characterized by severe, invasive infections with *Streptococcus pneumoniae*. Using the PFGE technique a genetic linkage was found between two *S. pneumoniae* serotype 14 isolates causing arthritis and meningitis at 3 and 5½ years of age, respectively, in a boy with IRAK-4 deficiency. This finding suggested that patients with IRAK-4 deficiency may harbour persistent strains of pneumococci. Alternatively, reinfection with strains from close contacts of the patient might cause recurrent invasive disease. It is proposed that eradication of pneumococci from the nasopharynx, and immunization of household contacts may prevent recurrent infection in IRAK-4-deficient patients.

Received 2 November 2006

Accepted 22 February 2007

Case report

An 8-year-old boy developed purulent arthritis of the right hip joint and meningitis at 3 and 5½ years of age, respectively. Both infectious episodes were caused by *Streptococcus pneumoniae*, and were treated with third generation cephalosporins; the patient recovered from these infections without complication. The genetic and immunological features are described in detail elsewhere (Ku *et al.*, 2007). Genetic analysis unveiled compound heterozygous mutations of the interleukin-1 receptor-associated kinase (IRAK)-4 gene in the patient, and heterozygosity for these mutations in the parents. Western blotting revealed a complete lack of IRAK-4 protein in fibroblast cells from the patient. Immunological studies showed severe anti-polysaccharide antibody deficiency after both natural infection and immunization with pneumococcal vaccines. After recovery from the second episode of invasive pneumococcal disease (IPD), intravenous immunoglobulin replacement therapy was started with a dose of 400 mg kg⁻¹ month⁻¹. During the past three years the patient has been free of IPD or other severe infection.

The relatedness of the pneumococcal isolates cultured from the hip joint and cerebrospinal fluid was studied in detail.

Abbreviations: IPD, invasive pneumococcal disease; IRAK, interleukin-1 receptor-associated kinase.

As both pneumococcal isolates were from sterile sites, identification was based on colony morphology, an optochin sensitivity test and solubility in 10% sodium deoxycholate. In addition, we confirmed the identity of the isolates by PCR amplification of the autolysin (*lytA*) gene (Nagai *et al.*, 2001). Conventional serotyping was carried out by coagglutination using antisera obtained from the Mast Group. The serotypes were also determined by type-specific PCR (Brito *et al.*, 2003). Both *S. pneumoniae* isolates proved to be serotype 14 (Fig. 1a).

Antibiotic susceptibility testing (determination of MIC) was performed by the agar dilution method. ATCC 49619 and NCTC 7465 strains were used as controls. As shown in Table 1, antibiotic susceptibility of the two serotype 14 isolates from the patient was identical (with a negligible difference in sensitivity to vancomycin), and different from that of two serotype 14 isolates from patients with pneumonia and one from a child with pneumococcal bacteraemia. The presence of four resistance-determinant genes [*ermB*, *mefE/A*, *ermA* and *ermTR*], was tested by PCR (Dobay *et al.*, 2005a). Both isolates carried the *ermB* macrolide-resistance determinant, which is the most frequent cause of macrolide resistance in Hungary (Fig. 1b; Dobay *et al.*, 2005a). The two strains were negative for *mef* genes. These data further suggested that the two isolates were identical. To define genetic relatedness, PFGE

Fig. 1. Molecular characteristics of the two *S. pneumoniae* serotype 14 isolates. (a) Serotype determination by PCR of the two pneumococcal isolates (lanes 1, 2) with serotype 14-specific primers (product size 268 bp). Numbers on the left indicate molecular mass sizes (bp). M, 100 bp ladder. (b) Detection of macrolide-resistance genes by PCR. Lanes 1 and 2, the two pneumococcal isolates; lane 3, *ermB* control (639 bp); lane 4, *mef* control (348 bp). M, 100 bp ladder. (c) PFGE patterns of genomic DNA generated by *ApaI* digestion for the two pneumococcal isolates (lanes 1, 2). Numbers on the left denote molecular mass sizes (kb). M, λ ladder.

was performed as described by Hall *et al.* (1996). Plugs of chromosomal DNA were digested with 30 U *ApaI* for 6 h at 37 °C. The fragments were separated on 1% agarose gel using 2 and 30 s pulse times, for 22 h at 14 °C. Next, the gels were stained with ethidium bromide. We found that the PFGE banding patterns of the two isolates were identical (Fig. 1c).

Discussion

IRAK-4 deficiency is a recently discovered primary immunodeficiency disorder characterized by recurrent, invasive infections with *S. pneumoniae* (Picard *et al.*, 2003). All known patients with IRAK-4 deficiency have had at least one episode of IPD, but recurrent infections caused by the same serotype have not been reported before (Yang *et al.*, 2005). We present here a patient with IRAK-4 deficiency and recurrent invasive infections caused by *S. pneumoniae* serotype 14. In addition, we provide

evidence of the genetic linkage between the *Pneumococcus* serotypes isolated from the patient at 3 and 5½ years of age.

Recurrent IPD has been defined as a subsequent positive culture of *S. pneumoniae* from any normally sterile site obtained >30 days after the initial positive culture (King *et al.*, 2003). Recurrent infection may represent relapse or may be due to new infection. We recently reported that serotyping is insufficient to assess the prevalence of relapses, since pneumococcal strains of the same serotype may be genetically unrelated (Dobay *et al.*, 2005b). Therefore, molecular typing, as an additional tool, must be performed to define relatedness between invasive pneumococcal isolates of the same serotype.

There have been no previous attempts to analyse *S. pneumoniae* isolates that cause recurrent invasive infections in patients with IRAK-4 deficiency. We report here what is to the best of our knowledge the first case of an

Table 1. Antibiotic sensitivity of *S. pneumoniae* serotype 14 isolates

Sample no.	Origin of isolate	Diagnosis	Age (years)	Serotype	lytA	ermB	MIC (µg ml ⁻¹)‡							
							Pen	Cefo	Clin	Teli	Cipro	Moxi	Vanco	Line
16572	Debrecen*	Arthritis	3	14	+	+	0.5	0.5	0.125	0.06	1	<0.03	1	0.5
23176	Debrecen*	Meningitis	5.5	14	+	+	0.5	0.5	0.125	0.06	1	<0.03	0.5	0.5
4764	Budapest	Pneumonia†	3.5	14	+	+	1	1	32	0.25	1	0.25	0.25	1
538	Győr	Pneumonia†	62	14	+	+	1	1	4	0.125	1	0.125	0.5	0.5
17208	Budapest	Bacteraemia	1.5	14	+	NT	0.125	0.125	64	0.03	2	0.25	0.5	0.5

NT, Not tested.

*Isolates from the patient described in the paper.

†*S. pneumoniae* isolates from sputum from the two patients with pneumonia.

‡Cefo, cefotaxime; cipro, ciprofloxacin; clin, clindamycin; line, linezolid; moxi, moxifloxacin; pen, penicillin; teli, telithromycin; vanco, vancomycin.

IRAK-4-deficient patient with recurrent episodes of IPD caused by genetically identical isolates. This observation provides the most compelling evidence that patients with this immunodeficiency disorder present with a unique susceptibility to invasive pneumococcal infections. Long-term survival strategies of pneumococci may exist, and may be different in immunocompetent and immunodeficient individuals. In particular, pneumococci may survive in tissue compartments of IRAK-4-deficient patients for years because of the impaired antibody-mediated and innate immunity. Long-term survival of intracellular pathogens in mononuclear phagocytes is well known. Data from our lab suggest that group B streptococcus type III, an extracellular pathogen, may survive *in vitro* in resident monocyte-derived macrophages, and macrophage activation is needed to kill ingested bacteria (Maródi *et al.*, 2000). Macrophage activation in IRAK-4-deficient patients may not be fully achieved by macrophage-activating agents, raising the possibility that these individuals may harbour streptococci in macrophages. Alternatively, recurrent IPD in patients with IRAK-4 deficiency may result from persistent nasopharyngeal carriage strains.

This report clearly indicates the need for prophylactic measures, including carrier detection and eradication of *S. pneumoniae* from the nasopharynx of the patient, as well as of household contacts. Active immunization of household contacts may reduce nasopharyngeal carriage and the likelihood of reinfection from family members. IRAK-4-deficient patients have an impaired antibody response to both polysaccharide and conjugate pneumococcal vaccines (Ku *et al.*, 2007). Therefore, passive immunization with intravenous immunoglobulin preparations containing anticapsular antibodies may provide protection by augmenting antibody-mediated opsonophagocytosis.

This case report also highlights the importance of serotyping and determination of DNA restriction patterns of pneumococcal isolates from patients with IRAK-4 deficiency in order to optimize the surveillance system for invasive disease caused by *S. pneumoniae*. Continued surveillance will be needed to define the extent to which infections by invasive pneumococcal strains (e.g. serotypes 4, 6B, 9V, 14, 18C, 19F and 23F) occur in patients with IRAK-4 deficiency and their implications for routine case management.

Acknowledgements

This work was supported by the Hungarian Research Fund (OTKA T49017 and F61665). We thank Drs J.-L. Casanova and C. Picard for helpful discussions.

References

- Brito, D. A., Ramirez, M. H. & de Lencastre, H. (2003). Serotyping *Streptococcus pneumoniae* by multiplex PCR. *J Clin Microbiol* **41**, 2378–2384.
- Dobay, O., Rozgonyi, F. & Amyes, S. G. B. (2005a). Molecular characterization of Hungarian macrolide resistant *Streptococcus pneumoniae* isolates, including 3 highly resistant strains with *mef* gene. *Int J Antimicrob Agents* **25**, 488–495.
- Dobay, O., Rozgonyi, F., Hajdú, E., Nagy, E., Knausz, M. & Amyes, S. G. B. (2005b). The relationship between serotypes and PFGE genotypes in isolates of *Streptococcus pneumoniae* from Hungary. *Clin Microbiol Infect* **11**, 673–676.
- Hall, L. M., Whitley, C., Duke, R. A. B., George, R. C. & Efstratiou, A. (1996). Genetic relatedness within and between serotypes of *Streptococcus pneumoniae* from the United Kingdom: analysis of multilocus enzyme electrophoresis, pulsed-field gel electrophoresis, and antimicrobial resistance patterns. *J Clin Microbiol* **34**, 853–859.
- King, M. D., Whitney, C. G., Parekh, F. & Farley, M. M. (2003). Recurrent invasive pneumococcal disease: a population-based assessment. *Clin Infect Dis* **37**, 1029–1036.
- Ku, C. L., Picard, C., Erdős, M., Jeurissen, A., Bustamante, J., Puel, A., von Bernuth, H., Filipe-Santos, O., Chang, H. H. & other authors (2007). IRAK4 and NEMO mutations in otherwise healthy children with recurrent invasive pneumococcal disease. *J Med Genet* **44**, 16–23.
- Maródi, L., Káposzta, R. & Nemes, É. (2000). Survival of group B *Streptococcus* type III in mononuclear phagocytes: differential regulation of bacterial killing in cord macrophages by human recombinant gamma interferon and granulocyte-macrophage colony-stimulating factor. *Infect Immun* **68**, 2167–2170.
- Nagai, K., Shibasaki, Y., Hasegawa, K., Davies, T. A., Jacobs, M. R., Ubukata, K. & Appelbaum, P. C. (2001). Evaluation of PCR primers to screen for *Streptococcus pneumoniae* isolates and β -lactam resistance, and to detect common macrolide resistance determinants. *J Antimicrob Chemother* **48**, 915–918.
- Picard, C., Puel, A., Bonnet, M., Ku, C. L., Bustamante, J., Yang, K., Soudais, C., Dupuis, S., Feinberg, J. & other authors (2003). Pyogenic bacterial infections in humans with IRAK-4 deficiency. *Science* **299**, 2076–2079.
- Yang, K., Puel, A., Zhang, S., Eidenschenk, C., Ku, C. L., Casrouge, A., Picard, C., von Bernuth, H., Senechal, B. & other authors (2005). Human TLR-7, -8, and -9-mediated induction of IFN- α/β and - λ is IRAK-4 dependent and redundant for protective immunity to viruses. *Immunity* **23**, 465–478.