—Report on Experiments and Clinical Cases—

Study of T Serotypes and Emm Genotypes of *Streptococcus pyogenes* in Children with Pharyngitis and Tonsillitis

Toshinari Okabe[1,2], Sachiyo Takeda[1,2], Masatoshi Hida[1,2] and Tomomi Narisada[3]

[1]Division of Reproductive Medicine, Perinatology and Gynecologic Oncology, Graduate School of Medicine, Nippon Medical School
[2]Department of Pediatrics, Nippon Medical School Tama Nagayama Hospital
[3]Clinical Laboratory, Nippon Medical School Tama Nagayama Hospital

Abstract

Streptococcus pyogenes, or group A Streptococcus (GAS), causes superficial infections of the upper respiratory tract that manifest as diseases such as pharyngitis and tonsillitis. T serotypes, emm genotypes, and the antimicrobial susceptibility of GAS isolated from the pharynges of patients with pharyngitis and tonsillitis were studied. The two most common T serotypes were T12 (10/25: 40%) and T1 (7/25: 28%), and the two most common emm genotypes were emm12 (12/27: 44%) and emm1 (7/27: 26%). Good correlation was observed between these T serotypes and emm genotypes.
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Key words: *Streptococcus pyogenes*, T serotypes, emm genotypes, pharyngitis, tonsillitis, children

Introduction

Group A Streptococci (GAS) are pathogenic bacteria commonly associated with pharyngitis, tonsillitis, bronchitis and pneumonia, and often lead to sequelae such as acute glomerulonephritis and rheumatic fever. Early diagnosis and treatment of GAS infections is therefore essential in preventing these diseases.

The type-specific M protein found on the GAS cell surface is resistant to phagocytic activities of polymorphonuclear leukocytes, which makes it a significant pathogen[1].

GAS is one of most common human pathogenesis. The M-protein, which is the surface protein of the cell wall and an important virulence factor of GAS. M-protein on the cell wall have documented by the detection of M-protein gene (emm gene). GAS is classified by serological or immunological techniques and the classification is important on the epidemiological investigation of GAS.

In this study, we aimed to clarify emm type distribution and T serotype in South erea of Tokyo Tama city.

To identify the types of bacteria that cause pharyngitis and tonsillitis in pediatric outpatients, GAS strains were isolated from pharyngeal swabs and their type specificity was examined.

Subjects and Methods

The 27 study subjects were outpatients aged between 1 and 9 years visiting Nippon Medical

Correspondence to Toshinari Okabe, Department of Pediatrics, Nippon Medical School Tama Nagayama Hospital, 1–7–1 Nagayama, Tama, Tokyo 206–8512, Japan
E-mail: tokabe@nms.ac.jp
Journal Website (http://www.nms.ac.jp/jnms/)

Table 1 Disease, treatment, and underlying diseases

	Age	Sex	Disease	Treatment	Underlying disease
1	6Y9M	F	URI	CDTR-PI	
2	9Y9M	M	URI	CFTM-PI	Bronchial asthma
3	8Y1M	F	URI	CDTR-PI	
4	8Y	M	URI	CFPN-PI	
5	7Y1M	F	URI, Diarrhea	CDTR-PI	
6	1Y6M	M	URI	CFTM-PI	Atopic dermatitis
7	9Y1M	F	URI, Bronchitis	CFTM-PI	Bronchial asthma
8	5Y2M	M	URI	CFPN-PI	
9#	7Y4M	M	URI	AMPC	
10	7Y10M	F	URI	CFTM-PI	
11	3Y8M	M	URI	AMPC	
12	6Y4M	M	URI	CFTM-PI	
13	6Y11M	M	URI	CFPN-PI	
14	3Y8M	F	URI	AMPC	Bronchial asthma
15	1Y11M	M	URI	CDTR-PI	
16	5Y3M	F	URI	CFTM-PI	
17	2Y3M	F	URI	CFTM-PI	Bronchial asthma
18	4Y6M	M	URI	CDTR-PI	
19	7Y9M	M	URI	CFPN-PI	
20	9Y11M	M	URI	AMPC	
21	8Y9M	F	URI	CFPN-PI	
22#	7Y7M	M	URI	CFPN-PI	Bronchial asthma
23	6Y5M	M	URI	CDTR-PI	
24	2Y11M	M	URI	AMPC	Bronchial asthma
25	1Y10M	M	URI	CAM	Bronchial asthma
26	4Y5M	F	URI	CXD	
27	6Y6M	F	URI, Diarrhea	CDTR-PI	

URI: Upper Respiratory Infection (Pharyngotonsilitis), CDTR-PI: cefditoren pivoxil, CFTM-PI: cefteram pivoxil, CFPN-PI: cefcapene pivoxil HCLhydrate, AMPC: amoxicillin, CTRX: ceftriaxone sodium, CAM: clarithromycin, CXD: cefroxadine
9 and 22: same patient

School's Tama Nagayama Hospital (Tama City, southern Tokyo) during the seven-month period May through November, 2008 with principal complaints of sore throat and fever, and in whom pharyngitis or tonsillitis was clinically diagnosed. Pharyngeal swabs were tested for GAS infection with a point-of-care testing kit (Rapid Testa Strep A, Sekisui Medical, Tokyo, Japan). Samples were also smeared on 5% sheep blood agar plates (Eiken, Tokyo, Japan) and cultured at 37℃. Beta hemolytic colonies were then subcultured and tested with a group-specific latex agglutination kit (Eiken, Tokyo, Japan) to identify GAS strains, and were preserved in glycerol at −80℃ for further testing.

T serotyping was performed with T-typing antisera (Denka Seiken, Tokyo, Japan), and M genotyping was done by sequence analysis of specific regions of the M protein gene (emm). Genes encoding M protein and M-like protein (emm) were amplified by polymerase chain reaction (PCR) according to the procedure reported by Beal et al.[2], and the Center for Disease Control and Prevention (CDC) database was used to identify the base sequences.

Results

The subjects' characteristics (age, gender, clinical diagnosis, and antibiotics administered) are listed in **Table 1**. Of the 27 subjects, 16 were boys and 11 girls. In addition to pharyngitis or tonsillitis, 7 of them had bronchial asthma, and 1 had atopic dermatitis.

The main method of treatment was penicillin or

Table 2 Lancefield group, T type, and emm type of streptococci isolated from patients' pharynges

	Lancefield group	T type	emm type
1	Group A	1	1
2	Group A	12	12.34
3	Group A	6	6.4
4	Group A	1	1
5	Group A	12	12
6	Group A	12	12.34
7	Group A	25	75
8	Group G	※	STG6792.3
9#	Group A	28	28
10	Group A	12	12
11	Group A	12	12.34
12	Group A	12	12
13	Group A	1	1
14	Group A	1	1
15	Group A	1	1
16	Group A	1	1
17	Group A	12	12
18	Group A	4	4
19	Group A	12	12
20	Group A	12	12
21	Group A	3	3.1
22#	Group A	12	12
23	Group A	4	4
24	Group A	1	1
25	Group A	UT	58
26	Group A	—	12.34
27	Group A	—	12

UT: untypable
9 and 22: same patient
※Group G

cephem antibiotics administration (**Table 1**). As shown in **Table 2**, GAS was detected in 26 of the 27 subjects, and group G streptococcus (GGS) was found in one subject. T serotyping was performed in 25 subjects, and emm typing in all 27. The most prevalent T types were T1 (7 cases) and T12 (10). One case each of types 3, 6, and 28 was found, and one was untypable.

M protein genotyping identified 7 cases of emm1, 11 cases of emm12, 2 cases of emm4, and one each of emm3, 6, 75, and 28. Overall, a significant correlation was observed between T and emm types.

The predominant GAS T-types were T12 (10/25, 40%) and T1 (7/25, 28%). T25, which has become increasingly prevalent in recent years, was found in one subjects. Similarly, emm typing identified 2 predominant types: emm12 (12/27: 44%) and emm1 (7/27: 26%). GGS was detected in Patient 8; the emm type was STG6792.3. The type T25 strains were genotyped as emm75. Case 9 and Case 22 were found in the same patient, who became re-infected 3 months after initially recovering. The T and emm types of this patient changed from T12/emm12 to T28/emm28, indicating that the second infection was not caused by the same strain as the first.

Discussion

None of the subjects in this study developed invasive GAS infection.

Ikebe et al. reported that in their study of invasive GAS infection the predominant emm genotypes were emm1 (42.7%), emm3 (11.8%), emm12 (7.3%), and emm28 (7.3%)[3]. Rogers et al. reported that emm1, 12 and 18 were the most frequently isolated emm types in invasive GAS infection, whereas emm1, 75, 28 and 4 types predominated in pharyngitis isolates, and that no significant association was observed between emm type and pharyngitis[4].

These reports are consistent with a report on emm types of GAS in Japan[5]. Although the association between age and emm type was not examined in the current study due to the relatively small sample size, Jaggi et al. reported that emm12 and 1 predominated in 3–6 year olds, while uncommon emm types increased in subsequent age groups (7–10, 11–14, and 15–18 year olds)[6]. Further studies are needed to examine the difference in genotypes between severe and mild forms of GAS infection in children and its association with age.

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