J. Hyg., Camb. (1983), **91**, 71–76
Printed in Great Britain

Type 49 *Streptococcus pyogenes*: Phage subtypes as epidemiological markers in isolates from skin sepsis and acute glomerulonephritis

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(*Received* 21 *February* 1983; *accepted* 14 *March* 1983)

SUMMARY

Studies of group A, M type 49 streptococci from England, Trinidad and Alaska indicate that isolates of this serotype often differ with respect to phage subtype from one geographical area to another, but are generally homogeneous in one place at one time. The findings support the conclusion that acute glomerulonephritis can be associated with a variety of phage subtypes of M type 49 streptococci.

In outbreaks of skin sepsis without nephritis in England, the phage subtypes of M type 49 streptococci isolated from skin lesions of meat handlers were the same as those recovered from skin lesions of non-meat handlers in the same community.

The findings on the Trinidad isolates suggest that M type 49 streptococci of one phage subtype may persist in a population for 9 years and may result in a second outbreak of acute glomerulonephritis.

In an Alaska Eskimo population in whom acute glomerulonephritis was occurring, most of the M type 49 isolates available for testing were of a single phage subtype. Equally prevalent in this population were group A streptococci that exhibited the same T antigen as the type 49 isolates but differed in their serum opacity reaction and phage subtype. This apparently related strain was not typable with available M antisera but showed functional evidence of M protein and is probably a new M type.

INTRODUCTION

For the past several decades M type 49 strains of group A streptococci (*Streptococcus pyogenes*) have been frequently associated with pyoderma and with acute glomerulonephritis. Since their first recognition in Minnesota (Kleinman, 1954; Updyke, Moore & Conroy, 1955; Wannamaker & Pierce, 1961), isolates of this serological type have been found in many parts of the world (Maxted, Fraser & Parker, 1967) and have reappeared in Minnesota 13 years later in association with a second outbreak of acute glomerulonephritis (Anthony *et al.* 1967).

The development of a phage typing system for type 49 streptococci has

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facilitated their division into subtypes based on lytic patterns (Skjold & Wannamaker, 1976). We have applied this phage typing method to M type 49 and related isolates recovered during outbreaks of skin sepsis in England, and during epidemics of acute glomerulonephritis in Trinidad and Alaska.

MATERIALS AND METHODS

The M type 49 streptococci from recent outbreaks in England were recovered from meat handlers and other patients with skin sepsis in Oxfordshire (Slack, Saunders & Mayon-White, 1982) and Cambridgeshire, from patients in a burns unit in Kent and from children with skin infections in a residential home in Lincolnshire.

The M type 49 streptococci isolated in Trinidad are from the collection of the Streptococcal Reference Laboratory, Central Public Health Laboratory, Colindale, England. They include previously examined isolates (Skjold & Wannamaker, 1976) from the acute glomerulonephristis epidemic of 1965–66 (Maxted, Fraser & Parker, 1967; Parker *et al.* 1968) and more recent isolates, from 1968 and from a second period of epidemic nephritis in 1975–6. Serologically related isolates from the 1975–6 period were also studied.

The Alaska isolates were recovered in 1975–81 from native Eskimo villages in which impetigo and acute glomerulonephritis were prevalent (Margolis *et al.* 1980); they included isolates confirmed in our Minnesota laboratory as M type 49 and serologically-related isolates.

All group A streptococci were examined for T, M and serum opacity reaction (SOR) antigens (Williams, 1958; Maxted *et al.* 1973) and were phage typed by a method previously described (Skjold & Wannamaker, 1976). Some isolates with serologically undetectable M protein were examined for evidence of functional M protein by rotation in blood from two or more normal human donors (Lancefield, 1959).

RESULTS

M type 49 isolates from four incidences of skin sepsis in England were examined (Table 1). There were no reports of acute nephritis in connection with these cases of skin infections. The isolates from Oxfordshire, comprising three isolates from abattoir workers, two from butchers, one from a chef and three from other patients with skin infections, were all phage subtype II (lytic pattern 1/3/4). Those from Cambridgeshire, which included three isolates from butchers, one from a fishmonger and four from other patients with skin sepsis, were all provisional phage subtype VI (lytic pattern 1/2/3/4). Most of the isolates from patients in the burns unit in Kent were the same phage subtype as the Oxfordshire streptococci. One of the isolates from Kent and all of the isolates from children with skin infections in a residential home in Lincolnshire were of the same phage subtype as the streptococci from Cambridgeshire.

The M type 49 isolates from a large 1965–6 epidemic of nephritis in Trinidad were confirmed as phage subtype III (lytic pattern 1/2/4) (Table 2). Most of the M type 49 isolates from a later (1975–6) outbreak of nephritis in Trinidad and all of those examined from an intervening year (1968) of infrequent nephritis were also phage subtype III. An additional five isolates from the 1975–6 outbreak (not included in Table 2) contained the T14 antigen associated with M49 strains (Maxted

Table 1. *M type* 49 *streptococcal isolates from four incidences of skin sepsis without nephritis in England*

Year(s)	Location	No. of isolates	Phage classification: Lytic pattern	Phage classification: Subtype
1980–1	Oxfordshire	9	1/3/4	II
1980	Cambridgeshire	8	1/2/3/4	VI*
1980	Kent	3	1/3/4	II
1980	Lincolnshire	3	1/2/3/4	VI

* New provisional phage type.

Table 2. *M type* 49 *streptococcal isolates from epidemic and non-epidemic periods of acute glomerulonephritis in Trinidad*

Year(s)	Nephritis	No. of isolates	Phage classification: Lytic pattern	Phage classification: Subtype
1965–6	Epidemic	13	1/2/4	III
1968	Infrequent	4	1/2/4	III
1975–6	Epidemic	10	1/2/4	III
		2	1/2/3/4	VI*

* New provisional phage type.

et al. 1967) but, in contrast to the M49 isolates, were SOR negative and non-typable with available phages. Although these five isolates were serologically negative for known M antigens,* they survived and multiplied in rotating human blood and thereby gave evidence of functional M protein.

In the Alaska Eskimo population with a high frequency of acute glomerulonephritis (Margolis *et al.* 1980), most of the M type 49 isolates were classified as phage subtype V (lytic pattern 1/4); two of the 13 M type 49 isolates available for study differed in that they were not typable with standard phages but were lysed by a new provisonal phage 5 (Table 3).

Twelve of the Alaska isolates exhibiting the T14 antigen of M49 streptococci were serologically negative for M type 49 and M type 14 (commonly associated with this T antigen) as well as for other recognized M types.† These 12 isolates (Table 3) gave functional evidence of M protein on rotation in normal human blood and probably represent a new M type. In contrast to the M49 isolates, they were negative for the serum opacity reaction, were uniformly not typable with available phages and were demonstrably lysogenic.

An additional five isolates of T type 14 (not included in Table 3) were serologically negative for known M antigens, were SOR negative and were negative for M protein on rotation in blood from three different normal donors. Two of these

* All five of these isolates were examined with an M antiserum for type 80 streptococci and found to be negative. T14, M80 streptococci have recently been identified in Britain, mostly among meat workers with skin sepsis (Fraser *et al.* 1977; Mayon-White & Perks, 1982).

† All 12 of these isolates were examined with an M antiserum for type 80 streptococci and found to be negative.

Table 3. *M type 49 and related streptococcal isolates from a nephritis-associated epidemic in Alaska*

No. of isolates		Antigen classification			Phage classification	
Total	From cases of nephritis	T	M	SOR	Lytic pattern	Subtype
11	5	14	49	+	1/4	V
2	1	14	49	+	None†	None
12	1	14	+*	−	None	None

* M positive by blood rotation (Lancefield, 1959) but serologically negative for M type 49 and other M antisera.

† These strains showed no lysis with standard phages (Skjold & Wannamaker, 1976) but were lysed by a new provisional phage 5.

isolates were of the same phage subtype (V) as most of the M type 49 isolates and were probably variants that had lost their M protein. Of the remaining three isolates, one was phage subtype IV (lytic pattern 1) and two were not typable with available phages.

In the Alaska population, M type 49 streptococci of phage subtype V were isolated from five of seven patients with acute glomerulonephritis (Table 3). M type 49 streptococci that were non-typable with available phages were recovered from one patient with this complication, and streptococci of the postulated new M type and non-typable with available phages were isolated from one case of acute glomerulonephritis.

DISCUSSION

Streptococcal skin sepsis in meat handlers and in poultry handlers has been described in several outbreaks in the United Kingdom and in the United States and has been associated with a variety of serological types of group A streptococci (Slack *et al.* 1982; Fraser *et al.* 1977; Mayon-White & Perks, 1982; Fraser *et al.* 1979; Working Group on Streptococcal Infection in Meat Handlers, 1979; Tsai *et al.* 1979; Barnham, Kerby & Skillin, 1982). We have shown here that the M type 49 streptococci isolated in two geographically distinct areas in England were of different phage subtypes (II and provisional VI), but within each area the M49 isolates were the same phage subtype irrespective of whether they were recovered from meat handlers or others with skin sepsis. Moreover, the phage subtypes of M type 49 isolates from two other geographically separate outbreaks in England not associated with meat handlers were (with one exception) also phage subtypes II and provisional VI, respectively. These phage typing results suggest that the type 49 isolates from outbreaks of skin sepsis in England are related to the geographical location and not to the occupation of the patients.

The phage subtyping data on the M type 49 isolates from Trinidad suggest that the same strain (with respect to both serological classification and phage type) was responsible for both of the epidemics of pyoderma-associated acute glomerulonephritis and probably persisted during the 9 year interval between the two periods of increased incidence of this kidney disease. In contrast, the two outbreaks of pyoderma-associated acute glomerulonephritis at Red Lake, Minnesota, separated

by 13 years, resulted from streptococcal infections with M type 49 strains of two different phage subtypes (IV and I) (Anthony *et al.* 1967; Skjold & Wannamaker, 1976). Thus, the findings on the isolates from Red Lake support the view that acute glomerulonephritis may also reappear in the same population after the appearance or introduction of a nephritogenic streptococcus of the same serologic type but different phage subtype.

Isolates from a nephritis-associated outbreak of streptococcal impetigo in an Alaska Eskimo population exhibited a common T protein, but were heterogeneous with respect to M typing, SOR and phage typing. The M type 49 isolates available for examination were all SOR positive, and almost all were phage type V. Equally prevalent were strains of the same T type (T14), but these strains were negative for M type 49 as well as other known M types and were SOR negative and non-typable with available phages. These latter strains grew well in rotating normal human blood and probably represent a new M type, although attempts to produce a specific M antiserum for this strain have been unsuccessful.

These and earlier studies (Skjold & Wannamaker, 1976) of M type 49 streptococci indicate that the phage subtypes of this serotype tend to be homogeneous within one epidemiological incident. Exceptions may be the M type 49 strains from Alabama (Dillon *et al.* 1967; Dillon, Derrick & Dillon, 1974) and from Czechoslovakia (Šramek *et al.* 1964) in which multiple phage subtypes were found (Skjold & Wannamaker, 1976). The patients with M type 49 pyoderma-associated nephritis in Alabama presented over a long period of time; this perhaps permitted the emergence of multiple phage types. This is in contrast to the sharply defined epidemics at Red Lake and in Trinidad. The identification of two phage subtypes (IV and V) found in isolates from a microepidemic of acute glomerulonephritis in a small town in Czechoslovakia is more difficult to explain. It is of interest that one of these phage subtypes (V) found in Czechoslovakia is the same as has been identified here in most of the cases of acute glomerulonephritis in Alaska.

The combined findings of the present and earlier studies indicate that acute glomerulonephritis can be associated with all of the known phage subtypes of M type 49 streptococci. Further studies are needed to determine whether the frequent and geographically diverse outbreaks of M type 49 and related streptococci conform to the cloning concept of the spread of strains with enhanced communicability or pathogenicity, as has been suggested for outbreaks of *Salmonella typhimurium* enteritis (Rowe *et al.* 1980).

The authors are indebted to W. R. Maxted, G. Colman and C. A. M. Fraser, Cross Infection Laboratory, Central Public Health Laboratory, Colindale, London, N.W.9, and to Richard T. Mayon-White, Public Health Laboratory, Oxford and J. Boissard, Cambridge for providing isolates from England and Trinidad and for serological identification or confirmation of some of the isolates. Some of the Alaska isolates were provided by Mary Anne Fitzgerald, Arctic Investigations Division, Center for Infectious Diseases, Centers for Disease Control, Anchorage, Alaska. This work was supported by a grant from the National Institute for Allergy and Infectious Diseases, National Institutes of Health, U.S. Public Health Service, Bethesda, Maryland (AI08724). Lewis W. Wannamaker was a Career Investigator of the American Heart Association.

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