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The serotyping of hospital strains of streptococci belonging to Lancefield group C and group G

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SUMMARY

A collection of more than 300 strains belonging to either Lancefield group C or group G was made. The cultures had been isolated either sporadically from patients with serious disease or as apparent clusters from various nosocomial outbreaks. T-protein antigens were sought. So far, nine distinct serotypes have been found among the group G streptococci and seven serotypes amongst the *Streptococcus equisimilis* (group C) strains. Of the sixteen serotypes, four were the original T-types 7, 16, 20 and 21 described by Griffith (1934). Because of the similarities of representatives of the two groups to Lancefield group A streptococci (Griffith, 1934; Maxted & Potter, 1967) a few strains not unexpectedly carried T antigens usually seen in group A streptococci. Using this scheme it has been possible to serotype 76% of *S. equisimilis* strains of human origin and 82% of group G streptococci from human clinical material. A small collection of group C and group G streptococci of animal origin could not be serotyped with the experimental T-antisera.

INTRODUCTION

The haemolytic streptococci belonging to Lancefield group C and group G can be isolated as commensals from the throat, from cultures of the umbilical stumps of neonates and occasionally from routine vaginal cultures. They are, however, also able to cause serious disease in man such as pneumonia, cellulitis or endocarditis (Mohr *et al.* 1979; Ancona, Thompson & Ferrieri, 1979). Asymptomatic existence in the upper respiratory tract may be followed by complications such as acute pharyngitis and lymphadenitis (Hill *et al.* 1969). In animal populations groups C and G streptococci have been shown to cause serious infections (McFadden & Boon, 1949; Biberstein, Brown & Smith, 1980).

There has been a steady increase in the numbers of group C and group G streptococci received in this laboratory for further examination. These include strains from systemic diseases. There have also been clusters of strains from what appear to be hospital outbreaks.

A provisional typing scheme has therefore been developed to test whether or not these organisms are epidemiologically related and also, if there is an association between a particular systemic disease and the serotype of the strain responsible. Several species of streptococci comprise each of the serological groups C and G.

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One species in each group, namely *Streptococcus equisimilis* in group C and the 'large-colony' variety or *S. canis* in group G have many similarities to the major human pathogen *S. pyogenes*, Lancefield group A. Cell-wall protein antigens, notably the T proteins and M proteins, have been described in all three species (Griffith, 1934; Lawal *et al.* 1982; Maxted & Potter, 1967).

The T-protein antigen, although not a virulence factor, is an important epidemiological marker in the serological classification of group A streptococci. Griffith's (1934) original classification of pyogenic streptococci into different T-types included types 7, 16, 20 and 21 which were eventually found not to belong to Lancefield group A but to be strains of either group C or group G. Simmons & Keogh (1940) subdivided these organisms with a scheme based on a combination of serological and biochemical characteristics but their strains are apparently lost with the exception of one minute colony type strain.

The M-protein antigens of strains of group G isolated in Nigeria have been studied by Lawal *et al.* (1982). The T-protein antigens are, however, easier to study and, as with the group A streptococci, it seemed appropriate to make the first subdivision on the basis of these antigens.

MATERIALS AND METHODS

Bacteria

The streptococci used in this study were mainly from hospitals in England, but some were received from overseas. They were either random isolates from different sources and implicated in a serious disease or they were possible causes of nosocomial outbreaks. Stock cultures of group C and group G streptococci were also examined. Totals of 17 group C strains and 12 group G strains isolated from animals were included in the study. Table 1 lists the vaccine strains used for the preparation of the experimental T-antisera.

Preparation of the antisera

T-typing sera were prepared in rabbits using trypsinized whole cell vaccines. Each vaccine strain was grown in 250 ml of Colindale Todd-Hewitt broth containing 1 % (v/v) of a 10 % (w/v) sterile solution of Difco '1/250' trypsin (McLean,

Table 1. *Vaccine strains used in the preparation of antisera*

Lancefield group C		Lancefield group G	
Strain no.	T-type	Strain no.	T-type
NCTC 4540*	7	NCTC 5969	16
NCTC 5370	20	R80/4327	PT 4327
NCTC 5371	21	R80/5007	PT 5007
R80/3722†	PT 3722‡	R80/5356	PT 5356
R80/4225	PT 4225	R80/6866	PT 6866
R80/5582	PT 5582	R80/7023	PT 7023
R81/1058	PT 1058	R80/7118	PT 7118
.	.	R81/1986	PT 1986
.	.	R81/3181	PT 3181

* NCTC = National Collection of Type Culture strains.
† R = Laboratory strains from the Streptococcus Reference Unit.
‡ PT = Provisional type number.

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1953). Incubation was at 30 °C for 24 h to enhance the production of the T-antigen. The cells were harvested, washed and resuspended in 25 ml of phosphate-buffered saline (pH 7·8, 0·1 M saline, 0·2 M phosphate) containing 0·5% trypsin and left at room temperature for 24 h. The cells were then washed six times in sterile physiological saline and finally resuspended in 17 ml of saline and 3 ml of a formalin solution (8% (w/v) formaldehyde in saline) and left at room temperature for 4 h. The formaldehyde content of the suspension was then reduced by centrifuging the suspension and replacing half the supernatant with saline. Rabbits were immunized by intravenous injections of 1 ml of the vaccine twice weekly for at least 4 weeks.

Absorption of the antisera

The antisera were absorbed with an equal volume of packed cells of an appropriate strain (Williams, 1958). Antibodies, for example, to the group G antigens were removed by absorption, repeated when necessary, with the culture, group G R80/3430 carrying the T antigen 25. Group C, R79/3540 with the T antigen 4, was used for all group C serum absorptions. The absorption suspensions were left at room temperature for 24 h or at 37 °C for 4 h. The absorbed antisera were then titrated against their homologous trypsinized vaccine strain by slide agglutination and then, finally, against a series of suspensions of known T-types for any cross-reactivity. Thiomersal (20 mg/l) was added as a preservative.

Serological methods

Cell suspensions were prepared by trypsin extraction (V. D. Allison, unpublished) as modified by Efstratiou (1980). Agglutination tests were performed by Griffith's method. Each antiserum was used at the highest dilution which gave a strong reaction. This varied between 1 in 500 to 1 in 10000 depending upon the serum.

RESULTS

Antisera were first prepared for the T antigens 7, 16, 20 and 21 and their apparent occurrence in only strains of Lancefield groups C and G was confirmed. But these sera agglutinated only about one-fifth of the strains tested. Rabbits were then immunized with strains that did not carry these antigens and a provisional set of 16 experimental sera was obtained. The vaccine strains selected were random isolates from cases of disease and from probable outbreaks of infections.

Overall, of the human isolates examined, 76% of group C streptococci (*S. equisimilis*) and 82% of group G could be serotyped (Fig. 1). There were 42 instances of cross-over between T antigens belonging to the two Lancefield groups. Certain strains carried T antigens belonging to the other group. This of course was also seen with the 24 strains of Lancefield groups C or G sharing the T antigens, 2, 4, 28 or 8/25/Imp 19 with strains of group A.

Group C streptococci (*S. equisimilis*)

As shown in Fig. 1 and from the data in Table 2 it was possible to subdivide provisionally the Lancefield group C (*S. equisimilis*) strains into 11 different serotypes. Some 24% were, however, not typable. Many of the *S. equisimilis* strains among the random isolates (Table 2) were recovered from blood cultures from

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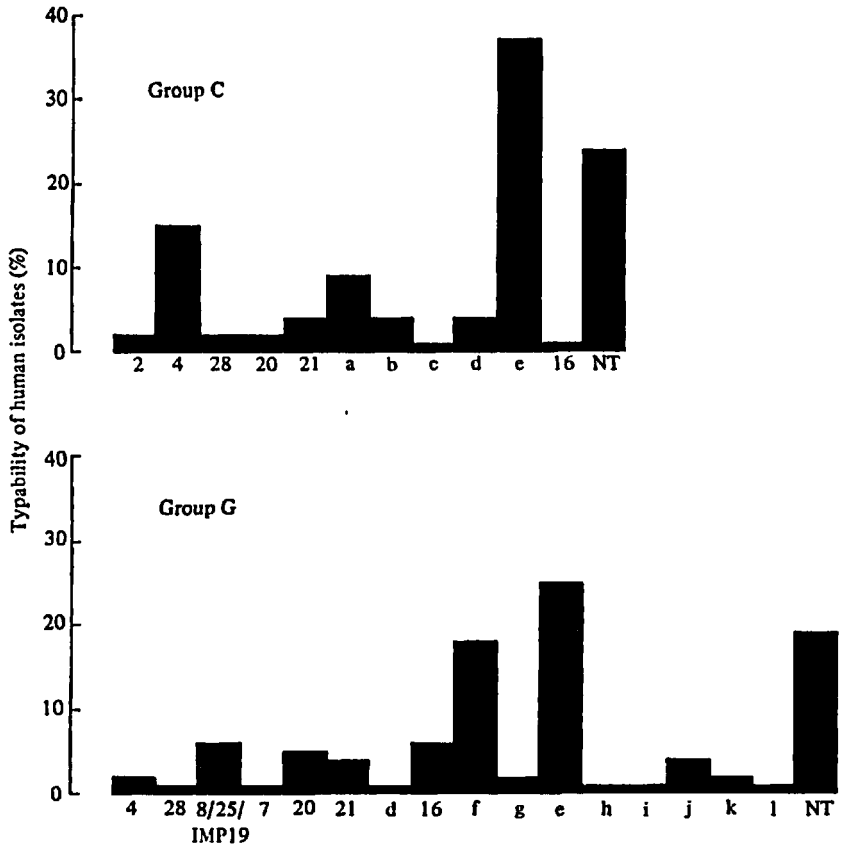

Fig. 1. Serological subdivision of Lancefield group C (*S. equisimilis*) and Lancefield group G by T-typing. NT = not typable, a = PT1058, b = PT3722, c = PT4225, d = PT5582, e = PT5007, f = 7/PT5356, g = PT4327, h = PT6866, i = PT7023, j = PT1986, k = PT3181, l = 20/PT3181.

cases of endocarditis or from throat swabs of patients with pharyngitis or tonsillitis. Of the 15 strains of group C isolated from blood cultures nine belonged to the provisional type '5007'. This is overall the commonest T-type in the collection. Three outbreaks of pharyngitis, presumably due to group C streptococci, were each caused by a single serotype. The strains from a single institution were serologically homogeneous. These included episodes of tonsillitis at two schools, with T-types PT1058 and PT3722 isolated separately at each school. The third outbreak was of pharyngitis in a military camp with T-type 4 being the serotype isolated from all throat swabs. Of the 111 cultures of Lancefield group C examined, 77 belonged to the species *S. equisimilis*. Of these strains eight were isolated from animals. There were 18 isolates of *S. equisimilis* from the outbreaks. A small group of strains belonging to other species of Lancefield group C were tested with the experimental sera but were not agglutinated (Table 3). These, with the exception of seven strains, were of animal origin.

Preliminary studies using trypsinized cells of other species of group C as immunogens were less successful (Table 4). Group antibody was produced in abundance. After absorption procedures were used to remove group antibody, slide

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Table 2. *Serotype distribution of single human isolates of* S. equisimilis *Lancefield group C*

Source	No. of isolates	Serotypes 2*	4*	28*	20	21	PT 1058	PT 3722	PT 4225	PT 5582	16	PT 5007	NT
Skin	6	.	.	.	.	.	.	.	.	.	.	.	6
Blisters	3	.	.	.	.	.	.	.	.	1	.	1	1
Wounds	4	.	.	.	.	.	.	.	1	1	.	1	1
Leg ulcers	6	.	.	.	.	.	.	.	.	.	.	4	2
Blood	15	1	.	1	.	.	.	1	.	.	.	9	3
Brain	1	.	.	.	.	.	.	.	.	.	.	1	.
Aspirates	3	.	.	.	.	.	.	.	.	.	.	1	2
Burns	1	.	.	.	.	.	.	.	.	.	.	.	1
HVS	2	.	.	1	.	.	.	.	.	.	.	.	1
Throat	17	.	1	.	1	4	3	.	.	2	1	3	2
CSF	1	.	.	.	.	.	.	.	.	.	.	.	1
Sputum	1	.	.	.	.	.	.	.	.	.	.	1	.
Urine	1	.	.	.	.	.	.	.	.	.	.	1	.
Ear	1	.	.	.	.	.	.	.	.	.	.	1	.
No information	7	.	3	.	.	.	.	.	.	.	.	4	.
Total	69	1	4	2	1	4	3	1	1	4	1	27	20

* T antigens characteristic of some strains of *S. pyogenes* (Lancefield group A).

Table 3. *Failure to detect T antigens amongst species of Lancefield group C streptococci other than* S. equisimilis *isolated from man*

Species	No. isolated	T-type
S. dysgalactiae	5	.
S. equi	2	.
*S. zooepidemicus**	5	.
S. equisimilis†	8	.
S. milleri‡	4	.

* These include three cultures from a family living on a farm.
† All isolates from animals.
‡ Isolates from humans of *S. milleri* with the Lancefield group C polysaccharide antigens.

Table 4. *Antibody response in rabbits to trypsinized immunogens of Lancefield group C streptococci*

Species	Vaccine strains	Group antibody	T antibody
S. equisimilis	See Table 1	+	+
S. dysgalactiae	NCTC 4669	+	−
	NCTC 4335	+	−
	NCTC 4671	+	−
S. equi	NCTC 9682	+	−
S. zooepidemicus	NCTC 6179	+	−

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agglutination tests with even the immunizing strains were all negative. The rabbits were then re-immunized and similar negative results obtained. This suggests that T-protein antigens may be absent from the other species of Lancefield group C.

Group G streptococci

Of the typable strains of group G streptococci, some 53% of the isolates were from hospital outbreaks. Cross-infection was suspected from the first and made more likely by the results of T-typing (see below). The data brought together in Table 5 show that the group G streptococci examined from single random isolates can be subdivided into at least fifteen T-types. Again, as with the group C streptococci, there were some instances of cross-over between particular T antigens of Lancefield groups A, C and G. These were 2, 4, 8, 25, 28, Imp 19, the types proposed here namely PT5007, PT5582 and Griffith's original types 7, 16, 20 and 21. The majority of group G strains were isolated from cases of severe diseases such as septicaemia, endocarditis, cellulitis and septic arthritis. Cultures were also recovered from patients with tonsillitis. Type associations between serious diseases and serotypes suggested that, in general, three T-types predominated among strains isolated from cases of septicaemia and endocarditis. Four-fifths of these 25 strains could be T-typed. A total of nine strains were agglutinated by both the type 7 and PT5356 sera, four by the PT5007 serum and three by the PT5582 serum. The largest groups of single isolates of group G examined were from blood, skin, leg ulcers and throat. Altogether, 73% of the random isolates were typable. This rate was increased to 82% by the inclusion of strains isolated from apparent outbreaks (Fig. 1).

Group G streptococci have been isolated recently from clusters of infections in several hospitals within the UK. They have, for instance been isolated from burns and plastic surgery units. From hospital A, many T-types were isolated. These are listed in Table 6. In the burns unit of this hospital, patients in two wards were involved in apparent outbreaks of group G infections. There appeared to be evidence of nosocomial spread in ward 1 with five isolations of T4 strains. The possibility of cross-infection in ward 2 was also considered. Strains from maternity units were also isolated. In one instance, cultures were isolated from a woman with a post-partum infection and representatives of the same type (7/PT5356) were isolated from a sample of bath water taken from the bath used by this patient. Two pairs of mother and baby related strains were seen. One pair of cultures was T-type 20 and the other 7/PT5356. An outbreak in an operating theatre of group G infections occurred at another hospital with serotype 16 isolated from all sources including environmental samples from the floor, floor mats and the footwear of staff. A total of 23 strains were isolated from leg ulcers but only 12 could be typed with the existing set of antisera.

As with group C, those group G strains isolated from animals could not be typed.

DISCUSSION

Comparatively little work has been done on the typing of human isolates of streptococci of Lancefield group C and group G, since the original work of Griffith, almost fifty years ago. This study is hopefully an extension of Griffith's original

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Table 5. *Serotype distribution of non-related human isolates of Lancefield group G streptococci*

Source	No. of isolates	Serotypes														
		28	8/25/ Imp 19	7	20	21	PT 5582	16	7/PT 5356	PT 4327	PT 5007	PT 6866	PT 7023	PT 3181	20/PT 3181	NT*
Skin	20	.	2	1	1	1	.	.	5	1	4	.	.	.	.	5
Blisters	3	.	.	.	.	.	.	1	.	.	2	.	.	.	.	0
Wounds	10	.	.	.	2	1	.	1	2	.	.	.	.	.	.	4
Leg ulcers	23	2	5	.	.	.	.	1	1	.	3	.	.	.	.	11
Blood	25	.	1	.	.	1	3	.	9	.	4	.	.	1	1	5
Biopsy site	1	.	1	.	.	.	.	.	.	.	.	.	.	.	.	0
Aspirates	5	.	2	.	.	.	.	1	1	.	.	.	.	.	.	1
Ear	4	.	.	.	1	.	.	.	1	1	.	.	.	.	.	1
Nose	3	.	.	.	.	.	.	.	1	.	2	.	.	.	.	0
Throat	20	1	.	.	1	.	.	.	3	2	4	.	1	1	.	7
Abscesses	5	.	.	.	1	.	.	.	1	.	1	.	.	.	2	0
Burns	1	.	.	.	.	.	.	.	.	.	1	.	.	.	.	0
Urine	1	.	.	.	.	.	.	.	.	.	1	.	.	.	.	0
Vagina	2	.	.	.	.	.	.	.	1	.	.	.	.	.	.	1
Umbilicus	1	.	.	.	.	.	.	.	1	.	.	.	.	.	.	0
No information	7	1	1	.	1	.	.	2	1	.	.	1	.	.	.	0
Total	131	4	12	1	7	3	3	6	27	4	22	1	1	2	3	35

* Not typable.

Table 6. *Origin and serotype distribution of clusters of Lancefield group G streptococci from apparent outbreaks*

Apparent outbreaks	Source	No. of isolates	T-type
Eye infections in one ward	Eyes	4	PT 5007
Self infection	Eye, finger	2	16
Operating theatres	Nose or throat of staff plus environmental isolates	6	16
School A	Nose, throat	5	3; type 21 2; PT 4327
Hospital A			
Burns Unit:			
Ward 1	Wounds	5	4
Ward 2	Wounds	19	5; 7/PT 5356 1; PT 5007 1; type 4 10; PT 1986 2; NT
Maternity Unit:			
Patients 1–4	Wounds	4	20; 21; PT 5007 & type 28
Patient 5	Perineum, bath water, bath water swab, toilet seat	4	7/PT 5356
Hospital B			
Ward 1	Wounds	1	21
Ward 2	Wounds	1	7/PT 5356
Ward 3	Wounds	2	PT 3181
Ward 4	Throat, nose, hairline	7	2; 7/PT 5356 5; PT 5007
Hospital C			
Two patients in one ward	Ulcers, wounds	2	7/PT 5356
Hospital D			
Special care baby unit	Infected umbilicus, septic spots	2	PT 5007
Hospital E			
Maternity Unit:			
mother; baby	HVS; nose	2	7/PT 5356
patient	HVS, urine	2	7/PT 5356
Hospital F			
Maternity Unit			
mother; baby	HVS; rectal swab, gastric aspirate and ear	4	20
Hospital G			
Patients in one ward	Impetigo, leg ulcer	2	NT
Hospital H*			
Burns Unit	Raw areas & environment	44	26; PT 5007 18; six other types

* Survey of group G streptococcal infection in a burns unit.

work and the system developed should be useful for serotyping clinical isolates, either random strains or more particularly as clusters.

Experience with T-typing of group A streptococci has shown that this method, used alone, will effectively indicate that an outbreak is occurring. For example, in the United Kingdom, the strains recovered from an outbreak of scarlet fever

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in a school and carrying the M-type 3 antigen will also have the T-type 3 antigen. For this reason some reference laboratories use T-typing alone for the subdivision of group A streptococci. But, for further study of these strains, as with Lancefield group A, examination for possible M antigens could be the definitive procedure.

The T-protein antigens of Lancefield group C and group G streptococci were found to be excellent immunogens. The titres of agglutinating sera ranged from 1 in 500 to 1 in 10000. It is unusual to find such high titres when group A strains are used as vaccines. In parallel with this there were also unfortunately high titres of group-specific antibody. Heavy absorption procedures were required. Taken together these results suggest that future experiments should explore the use of purified T proteins as immunogens and this is being done.

Many strains were isolated from blood cultures with the provisional types 5007 and the complex 7/5356 being the main serotypes for both Lancefield group C and group G. Further absorption of the sera could perhaps eliminate these cross-reactions between strains belonging to the different groups, but it does not appear necessary at present for epidemiological studies. It is noteworthy, however, that the T-type 7 antigen occurred alone in group C strains and in association with the PT5356 antigen in group G strains bar one.

It is suggested that further work on the preparation and perhaps purification of anti-T sera would extend this provisional serotyping scheme to strains presently untypable. That this could be worthwhile is indicated by the many strains isolated from leg ulcers that could not be typed. As far as is known the T antigens of the group A streptococci are not directly related to the virulence of these organisms and there is as yet no evidence to suggest whether or not the T antigens of the streptococci of group C or group G inhibit phagocytosis. By analogy, this would seem unlikely. Any exploration of mechanisms of pathogenicity would probably need to be preceded by an examination of these organisms for M antigens and preliminary work along this line has begun.

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