SHORT ARTICLE

A new scheme for serotyping group-G streptococci

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Summary. Ten antisera, prepared against nine selected strains of group-G streptococci and a strain of group-A streptococci of M-type 12, were used to serotype 102 isolates of group-G streptococci by means of precipitation reactions between the sera and hot-acid extracts of the streptococci. Fifty-six (54.9%) of the streptococci could be typed; eight serotypes were identified, of which type VIII was the most common (18.6%). The M12 and the R28 antigens, previously recorded in group-G streptococci, were not detected. The type antigens were trypsin sensitive and resembled the M antigens of group-A streptococci.

Introduction

Group-G streptococci are commonly isolated from the throat and from skin lesions in some tropical countries (Ogunbi, 1971; Valkenburg *et al.*, 1972; Ogunbi *et al.*, 1974a and b; Belcher *et al.*, 1975). Members of this group are known to cause disease in man, including endocarditis (MacDonald, 1939), epidemic pharyngitis (Hill *et al.*, 1969) and neonatal septicaemia (Baker, 1974). Serological typing of group-G streptococci by slide-agglutination tests for their T antigens has been reported (Laughton and Davies, 1957; Hill *et al.*, 1969; Efstratiou, 1983).

We have described the detection in group-G streptococci of several type antigens resembling the M antigens of the group-A streptococci (Lawal *et al.*, 1982); now we present an extension of our precipitation-typing system and give more information about the M-like antigens of group-G streptococci.

Materials and methods

Streptococci

The following members of group G isolated in our laboratory were used for the production of antisera: the six type strains previously described (nos. 28, 25, 19, 16, 21 and 18, representing respectively types I, II, III, IV, V and VI; see Lawal *et al.*, 1982) and two further strains (34/25/16/06 and 117/2968) corresponding to new types, respectively VII and VIII. In addition, Dr C. A. M. Fraser of the Streptococcus Reference Laboratory, Colindale, London, had provided us with a group-G strain (R51/755) which possesses the R28 antigen (Maxted, 1949), and a group-A, M-type 12 strain, because this M antigen has been encountered in group G (Maxted and Potter, 1967); sera were also made against these. A collection of 102 group-G isolates from throat swabs and skin lesions in Nigeria was examined for type antigens.

Serological methods

These have been described previously (Lawal *et al.*, 1982), and included: preparation of typing sera in rabbits; extraction of streptococci with hot HCl; precipitation tests for type antigens in agarose gel on microscope slides; complement-fixation tests for M-associated protein (MAP; see Widdowson *et al.*, 1971); and direct and indirect bactericidal tests in fresh human blood.

Trypsinisation of cultures

This was performed as described by Lancefield (1962).

Virulence and protection tests

These were made in 3-week-old laboratory mice as described by Maxted and Potter (1967). Inocula of 0.5 ml of an overnight culture in Todd-Hewitt Broth (Difco), undiluted, and diluted 1 in 10 and 1 in 100, were injected intravenously into the tail veins (six mice per dilution), and the animals were observed for deaths for 7 days. In the protection tests, 0.25 ml of undiluted rabbit antiserum of homologous type was given to each of six mice 24 h before challenge with 0.5 ml of a 1 in 10 dilution of culture. Controls with antiserum of heterologous type were included.

Results

The vaccine strains for the new types VII and VIII were chosen because they gave high titres in the complement-fixation test for MAP (respectively 160 and 320) and they did not react in the precipitation test with antisera for the six previously described serotypes. Post-immunisation sera were absorbed with strains of heterologous type to remove group antibody (Lawal *et al.*, 1982) and then gave precipitation reactions only with the homologous vaccine strain. They did not react with the group-A, M12 or the group-G, R28 serum.

Hot-acid extracts of 102 group-G isolates were tested in the precipitation test with the enlarged set of typing sera

Table I. Distribution of serotypes among 102 strains of group-G streptococci

Serotype number	Number (and percentage) of the indicated serotype
I	5 (4.9)
II	2 (2.0)
III	10 (9.8)
IV	6 (5.9)
V	2 (2.0)
VI	7 (6.9)
VII	5 (4.9)
VIII	19 (18.6)
Untypable	46 (45.1)

(table I); 56 of them (54.9%) fell into one of the eight serotypes. One of the new types (VIII) proved to be the most common, forming 18.6% of the total. None of the strains gave reactions with the M12 or the R28 serum.

The acid extracts were also tested for complement fixation against a standard dose of human anti-MAP serum (Widdowson *et al.*, 1971). The MAP antigen was detected in all but 11 (10.9%) of the cultures, but titres were low (40 or less) in 35 of them (34.2%). All the cultures in which a type-specific antigen had been detected had an MAP titre of 80 or more; the association of these characters was significant (χ^2 35.76; $p < 0.01$).

Trypsinisation of the hot-acid extracts of each of the vaccine strains abolished the precipitation reaction with its homologous-type antiserum; this indicated that the type antigens were proteins.

The eight vaccine strains were tested in direct bactericidal tests in fresh blood from two donors (A and B). All showed a > 100-fold increase in numbers in the blood of at least one of the donors, but strain V showed a

somewhat smaller increase in the blood of donor A and strains I and VII did not multiply in the blood of donor B. In indirect bactericidal tests (Maxted, 1956) in the blood of donor A, the multiplication of all of the vaccine strains was inhibited partially (multiplication not in excess of $\times 5$) or completely (no multiplication) by the corresponding rabbit antiserum but not by heterologous sera.

Six of the eight vaccine strains showed some virulence for mice by the intravenous route, a 1 in 10 dilution of culture killing the following numbers (of six) mice: I, 4; III, 2; IV, 4; VI, 4; VII, 5; VIII, 4. Two of the strains (nos. II and V) were non-virulent; all six of the mice that received undiluted culture survived. In mouse-protection tests with a challenge dose of 1 in 10 culture, the rabbit antisera for types I, III and VII gave complete protection against death, and the serum for type VIII gave partial protection; but the serum for type IV was not protective.

Discussion

The addition of two typing antisera to our set resulted in a considerable increase in the percentage of typable strains, from 28.8 to 54.9; this was mainly, but not entirely, attributable to type VIII, which formed 18.6% of the total. All the strains in which type antigens were detected gave high titres in the complement-fixation test for MAP; this confirmed the close association of this protein with the M-like antigens in the group-G streptococci, as with the M antigens in group-A streptococci.

The type antigens of group-G streptococci are destroyed by trypsin, and thus are proteins, like the group-A M antigens (Lancefield, 1962). The vaccine strains for types I-VI were shown by Lawal *et al.* (1982) to multiply in the blood of a single donor. This property was re-examined, for all eight vaccine strains, in the blood of two different donors. Though all the strains grew in the blood of at least one of the donors, some strains grew poorly or not at all in the blood of one or other of them. This may have been due to the presence of type-specific antibody in the blood samples.

Six of the eight type strains showed some virulence for mice by the intravenous route, but two were non-virulent. Rabbit antisera against five of the six mouse-virulent strains were tested for protective action in mice. Three of the five sera gave complete and one gave partial protection, but one was inactive in this respect.

We have identified type-specific antigens in more than one-half of a collection of Nigerian group-G streptococcal strains; these resemble the M antigens of group-A streptococci in being trypsin-sensitive proteins that confer antiphagocytic powers on the organism in human blood, and in being associated with MAP antigens. Like M-positive group-A streptococci, a number of the group-G streptococci—but not all of them—are mouse-virulent.

Table II. Relation of titre of M-associated protein (MAP) to typability

Titre of MAP	Number (and percentage) of strains	
	with the stated titre	and with an M-like antigen
0	11 (10.9)	0
10	8 (7.8)	0
20	8 (7.8)	0
40	19 (18.6)	0
80	22 (21.6)	22 (21.6)
160	28 (27.5)	28 (27.5)
320	6 (5.9)	6 (5.9)
Any	102 (100.0)	56 (54.9)

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