Absence of the Group-specific and the Cell-wall Polysaccharide Antigen in L-phase Variants of Group D Streptococci

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SUMMARY

The L-phase variants of group D streptococci, analysed in this study, were not only characterized by the absence of the cell-wall polysaccharide antigen, but also by the absence of the group D antigen, which is not a constituent of the cell wall.

INTRODUCTION

The absence of the cell wall in the L-phase variant of group A β -haemolytic streptococci has been shown by chemical and serological analyses (Sharp, Hijmans & Dienes, 1957; Panos, Barkulis & Hayashi, 1959) and by studies with the electron microscope (van den Hooff & Hijmans, 1959; Panos, Barkulis & Hayashi, 1960). The lack of this substance seems to be a general characteristic of these structures (for reviews see Klieneberger-Nobel, 1960; Kandler & Kandler, 1960), but little is known about the other components of the L-forms. The availability of group D streptococci in the L-phase enabled us to investigate this aspect, as Elliott (1959 and 1960) and Jones & Shattock (1960) had shown that in this species the group-specific polysaccharide is not a constituent of the cell wall.

METHODS

The group D streptococci used were obtained in the L-phase as described previously (Hijmans & Kastelein, 1960) and propagated in Brucella broth (Albimi Laboratories Inc., Brooklyn 2, N.Y., cat. no. A-114) + 2% agar (w/v) as the basal medium, with the addition of 4% (w/v) phosphate, 10% (v/v) horse serum and penicillin to a final concentration of 1000 units/ml. Two strains, designated as no. 27 and no. 30, could be grown as L-variants in a liquid medium of the same composition, but without agar. Some studies were also performed with group D strain D76, which was a gift from Dr S. D. Elliott of Cambridge; Dr E. H. Freimer had induced this strain to grow in the L-form by incubating the cocci in a penicillin gradient. L-form extracts were prepared from washed or untreated sediments, obtained by centrifugation of the cultures. To prevent lysis all washings were carried out with a 4% (w/v) phosphate solution. Routine extraction procedures were used such as hot HCl, formamide and streptomycin enzyme. Rabbits were immunized with formalin-killed suspensions of group D streptococci (strains no. 27 or 30) which had been shown to belong to the same type. Commercially available antisera proved to be unsatisfactory for our purpose. Type specific antisera were

prepared by absorbing with group D streptococci of serological type different from the immunizing strain; group specific antisera were prepared by absorbing with isolated cell walls of the immunizing strain.

Within group D streptococci type-specificity has not yet been defined satisfactorily. In this paper the type will be defined by the polysaccharide cell-wall antigen and the numbers refer to the different laboratory strains.

Ring precipitin tests were performed in capillary tubes with an internal diameter of 0.4 mm. To avoid false negative results due to antigen excess all tests were repeated with a 1/5000 dilution of the antigen.

Glucose and rhamnose were determined by using the cysteine + sulphuric acid method of Dische (1955). The presence of free glucose was tested for by the glucose oxidase reaction with 'Clinistix' (Ames Co., London).

RESULTS

The results (Table 1) show that the L-phase variants of the group D strains used in this study did not contain the group- or type-specific antigen. The production and subsequent release into the medium of these substances was made unlikely by the absence of a precipitate in the ring test performed with the supernatant fluid, and the absence of precipitate when the L-forms were grown in agar plates containing 10 % (v/v) of the appropriate antisera (Freimer, Krause & McCarty, 1959).

Table 1. *Absence of group-specific and type-specific polysaccharide in the L-phase variant of group D streptococci*

Antigen			Antisera		
			Group A	Group D	Group D (type)*
Strain	Group	Phase	Precipitin reaction		
AED	A	Coccal	+	—	—
GL8	A	Coccal	+	—	—
27	D	Coccal	—	+	+
30	D	Coccal	—	+	+
D76	D	Coccal	—	+	+
27	D	L	—	—	—
30	D	L	—	—	—
D76	D	L	—	—	—

* The group D strains were tested with antiserum, directed against their own cell-wall polysaccharide antigen.

The absence of group D antigen could not be explained by the properties of the medium, as strains in the coccal phase did synthesize the group D substance when grown in the basal medium + phosphate + horse serum.

The possibility was envisaged that simple haptens might be present, either in the L-phase variant or in the culture supernatant fluid. The former could be excluded, because formamide extracts did not contain either glucose or rhamnose. Release into the medium of a precursor of the group-specific antigen also was unlikely, because the hydrolysate of the supernatant fluid of L-type growth in broth gave a

negative glucose oxidase reaction, even in a tenfold concentration. The possibility of the presence of a precursor or a simple hapten of the cell-wall polysaccharide was studied by means of inhibition tests. Quantitative precipitin curves were made with a purified cell-wall polysaccharide preparation and its specific antiserum. Addition of the culture supernatant fluid to a series of these mixtures did not influence the antibody nitrogen content of the precipitates.

It can therefore be concluded that these L-phase variants of group D streptococci lack not only a cell wall, but also at least one other constituent, which is not part of the cell wall. When strain D76 reverted to the coccal state, the two antigens were again present. Attempts to bring about reversion of the other two strains were unsuccessful.

I am indebted to Dr S. D. Elliott (Cambridge) for strain D76 and to the Streptococcus Reference Laboratory, Central Public Health Laboratories, Colindale, London, for confirming some of our findings. It is a pleasure to record the assistance of Mrs K. Nikkels and Mr H. Radema.

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