Use of enzymes in typing group A β -haemolytic streptococci

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Summary. A series of 698 strains of group A β -haemolytic streptococci (GAS) isolated from children with streptococcal pyoderma was tested for production of serum opacity factor (OF) and nicotinamide adenine dinucleotide glycohydrolase (NADase). OF was produced by 37% of strains and 40% produced NADase. Classification based on various combinations of OF and NADase reactions showed that 58% belonged to enzyme group e1 and 34% to e2. Correlation with T and M types showed the possible use of this means of classification as an epidemiological marker for GAS. The specificity of such a system in the further classification of various T types of GAS in epidemiological studies, in the light of antigenic variation among M types, is described.

Introduction

Attempts have been made to identify group A streptococci (GAS) by enzyme markers, especially in situations where M typing cannot be successfully performed (Bergner-Rabinowitz and Ofek, 1974; Ivarsson and Christensen, 1977). Production of serum opacity factor (OF), an α -lipoproteinase, has been found to be M-type specific for the GAS that produce them (Top and Wannamaker, 1967; Widdowson *et al.*, 1971; Maxted *et al.*, 1973; Hallas and Widdowson, 1978). This property is utilised in OF typing. Another enzyme, nicotinamide adenine dinucleotide glycohydrolase (NADase), has also been shown to be a stable character for many M types (Lazarides and Bernheimer, 1957; Lütticken *et al.*, 1976). Using a combination of these reactions and correlating them with T typing, GAS can be classified and information on the M types circulating in a community can be obtained (Bergner-Rabinowitz and Ofek, 1974; Ivarsson and Christensen, 1977).

The present study reports observations on the characterisation of GAS associated with pyoderma, using enzymes, during a streptococcal surveillance study in a south Indian community medical programme, in Vellore, Tamil Nadu, India.

Materials and methods

Strains

A total of 698 strains of GAS isolated from skin lesions, throats and noses of children with pyoderma, seen during

a streptococcal surveillance programme conducted among rural school children in the south of India during 1975–1978 were characterised in this study.

Serum opacity reaction (SOR)

All the strains were tested for the production of opacity factor by the technique of Maxted *et al.* (1974), with certain modifications. Due to the unavailability of horse serum, preliminary OF testing was done with ox serum obtained locally. The preparation of serum for SOR followed the recommendations of Maxted *et al.* (1974). Thirty strains and control strains obtained from Dr W. R. Maxted, Public Health Laboratory Service, Colindale, London, were tested for OF production with ox serum. In a pilot study, results obtained with horse serum, in tests performed at Colindale, were identical to those with ox serum. Subsequently, all OF testing was performed with ox serum.

NADase testing

This was done according to the method of Lütticken *et al.* (1976). Positive and negative controls were included for each test set.

T typing

All strains were T-typed by accepted methods (Moody *et al.*, 1965), with antisera prepared and developed in this laboratory.

M typing

Because antisera were not available, M typing could not be performed; however, 30 strains were sent to Dr G. Colman, Colindale, for M typing.

Grouping of GAS by enzymes

The enzyme grouping was done as described by Bergner-Rabinowitz and Ofek (1974) and shown in table I.

Table I. Enzyme grouping of group A streptococci

Enzyme group	OF	NADase
e1	—	—
e2	+	+
e3	—	+
e4	+	—

Results

The figure shows the production of OF and NADase by various T types. T types 3/13/B3264, 4/28, 14 and 9 were poor producers of both NADase and OF whereas types 5/11/12/27/44, 8/25/Imp19, 6 and others were relatively strong producers of both enzymes. For a given T type the percentage positive for NADase was comparable to that for OF.

The correlation of various T types with enzyme groups is shown in table II; 403 (58%) of the 698 strains belonged to group e1, and 241 (35%) to group e2. Only 8% belonged to groups e3 and e4.

The correlation of 16 M-typable strains with their T types and enzyme patterns is shown in table III. The 16 strains represented 10 different M types and 7 T types. Eight belonged to group e2, five to e1 and three to e3.

Discussion

The continued emergence of new M antigens and

Table III. M type, T type and enzyme patterns of 16 M-typed strains of group A streptococci

T type	Number of strains	Enzyme group	M type
3/13/B3264	1	e1	52
3/13/B3264	2	e1	74
3/13/B3264	3	e3	<i>Trin. No. 2631</i>
4	1	e2	22*
4	1	e2	58
28	1	e1	30
11	2	e2	<i>Trin. Potter C</i>
8/25/Imp 19	2	e2	60
14	1	e1	39
9	2	e2	25

M-typing was done by Dr G. Colman, PHLS, Colindale, London.

*Typing by OF inhibition alone.

antigenic diversity among GAS have impeded classification methods for some time (Cleary *et al.*, 1979). The correlation between certain M and T types, recognised in the past, has lessened this problem to some extent (Parker, 1967; Maxted and Widdowson, 1972). However, a particular T antigen can be associated with more than one M antigen, whereas a single M antigen may be found with more than one T antigen (Parker, 1967; Wannamaker, 1970). Thus, a comprehensive set of M antisera has to be available for the complete identification of GAS, a situation which is outwith the scope and potential of most laboratories. Moreover, international reference centres do not supply them and they are not available commercially.

As a result of this, characterisation of GAS by enzyme profiles has been performed (Bergner-

Table II. Correlation of T types with enzyme patterns

T type	Number of strains	Enzyme group			
		e1	e2	e3	e4
3/13/B3264	192	132	44	13	3
4/28	113	89	17	4	3
5/11/12/27/44	91	35	42	6	8
8/25/Imp 19	60	16	39	4	1
9	78	53	24	1	0
14	61	35	24	1	1
6	42	27	13	1	1
1,2,22,23	61	16	38	7	0
Total	698	403 (57.7%)	241 (34.5%)	37 (5.3%)	17 (2.4%)

Rabinowitz and Ofek, 1974; Ivarsson and Christensen, 1977). Based on the production of NADase and OF, Bergner-Rabinowitz and Ofek (1974) classified 472 strains of GAS into 13 T patterns and 46 subtypes. Qualitative differences existed among the strains of each T pattern in relation to the site of infection, season and enzyme production. It was clearly demonstrated that enzyme production provides an important bacteriological tool for identification within T types. Moreover, enzyme classification enabled them to detect strains which, on further examination, could result in the identification of new M types.

In the present study, 698 GAS strains were divided into 11 T types. Irrespective of their sites of isolation, 58% belonged to group e1. This was notably so for T types 3/13/B3264, 4/28, 9, 14 and 6. In contrast only 35% of strains belonged to group e2, particularly types 5/11/12/27/44 and 8/25/ Imp 19; e3 and e4 occurred in only 8% of strains. These results are in marked contrast to those reported by Bergner-Rabinowitz and Ofek (1974) who reported an equal distribution of groups e2 and e3 (30% each) and a lesser number of group e1 (24%); 15% belonged to group e4. The pattern of T types was similar in the two studies but the distribution of enzyme groups was different. This indicates that the distribution of M types among these two vastly different populations varies considerably.

Correlation of T types, M types and enzyme patterns revealed interesting features regarding type specificities of these antigens (table III). Two M-typable strains of T4 had the same enzyme pattern, yet had two M antigens. Similarly strains of typable 3/13/B3264 showed two different enzyme patterns and three M antigens. The NADase reaction can differentiate GAS with the same T antigens. These results show that all these three antigenic specificities differ from each other, as has been reported by Cleary (1978). Moreover the genes coding for OF and M antigens have also been shown to be different.

This argues a case for more detailed characteris-

ation of T antigens in epidemiological studies. The present practice is to identify and describe these antigens as a complex and not as individual antigens. Differences in enzyme patterns such as e1 and e3 for the same complex 3/13/B3264 may represent differences in the three T antigens. A more detailed examination can reveal the true nature of the specificities of these antigens. Use of highly monospecific T antisera will then identify these antigenic differences, an important factor in the detection of strain variation of GAS.

The present study also confirmed an earlier observation that a larger number of known M types should be tested before a definite correlation with enzyme patterns could be made (Ivarsson and Christensen, 1977). This could be difficult as more new or hitherto unknown M antigens are being recognised. It is also known that antigenic drift occurs amongst GAS strains. Cleary *et al.* (1979) in studies on immunological specificities of type-specific markers such as T, M and OF antigens, noted that OF antigens can reflect differences between these markers and suggested that OF antigens are sensitive indicators of strain relatedness and differences. Subtypes can then be identified. These studies indicated that the identification of OF production in strains would produce more accurate and meaningful results than M typing. It is also known that genes coding for M proteins and OF antigens are distinct (Cleary, 1978). Our observation that M 22 antigen was detected by OF inhibition and not by conventional M typing supports this hypothesis.

In conclusion, this study shows that enzyme markers are useful indicators of variation in GAS strains in epidemiological studies and the techniques used are easier to perform than those required in the detection of M antigens.

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