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Streptococcal antibodies in patients with burn injuries

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SUMMARY

Serum samples from 14 patients whose burns had become infected with streptococci of groups A (11 patients), C (one patient) or G (two patients), and from 19 burned patients without bacteriological evidence of streptococcal infection were examined for anti-streptococcal antibodies. Tests were made for anti-streptolysin O (ASO), anti-hyaluronidase (AH), anti-deoxyribonuclease B (anti-DNAase B) and antibody against M-associated protein (MAP). Sera from the patients with streptococcal infections were also examined, when this was practicable, for 'bactericidal' (anti-M) antibody and for antibody against the opacity factor (OF) of the infecting serotype.

In patients infected with group A streptococci, the ASO response was generally poor, except in patients infected with strains of type T12/M12, and the AH response was rather similar, but most of the patients gave a rapid and vigorous anti-DNAase B response, except when the burn was small or colonization occurred very late. Antibody to the M and MAP antigens, and to OF (when the infecting strain formed this), was weak and transient, or absent, except in three of four patients infected with streptococci of type T12/M12.

INTRODUCTION

The policy at the Wessex Regional Burns Centre, designed specifically to reduce invasive infection of burns with *Pseudomonas aeruginosa* and other Gram-negative organisms, has been shown to allow frequent and often prolonged colonization with *Streptococcus pyogenes* (Wormald, 1970). Little information is available on the antibody responses of burned patients to infection with streptococci, and in particular it is not known whether such patients develop protective immunity against the M type with which they are colonized, or if a degree of toxic immunosuppression prevails that prevents this response.

In this study we have measured the development of antibody in patients with burn injuries colonized by various serotypes of group A streptococci, to three extracellular products (deoxyribonuclease B, streptolysin O and hyaluronidase) and to three cellular streptococcal antigens: M protein, opacity factor (OF) and M-associated protein (MAP).

MATERIALS AND METHODS

Streptococci. Nasal and throat swabs were taken on admission and subsequently if the patient suffered from sore throat. Routine serial swabbing of burn wounds and grouping of streptococci was done as described previously (Wormald, 1970). Group A strains were T typed (Griffith, 1934), M typed (Rotta *et al.* 1971) and tested for OF (Maxted *et al.* 1973) at the Streptococcus Reference Laboratory, Colindale.

Sera were collected from all burned patients weekly or more often by the Burns Research Laboratory, Odstock Hospital, whether the burns were colonised with streptococci or not. We tested 14 sets of sera from colonised patients (see Table 1) and 19 sets of sera from patients without bacteriological evidence of streptococcal colonisation of their burns.

Antibody tests

Serum samples were tested for the following antibodies as indicated, except when insufficient amounts were available.

ASO tests were performed on all sera by the spectrophotometric method of Gooder & Williams (1961).

Anti-DNAase B tests were done on all sera by the micro-method of Nelson, Ayoub & Wannamaker (1968).

AH tests were done on all sera by the micromethod of Murphy (1972), with reagents from Difco Ltd, West Moseley, Surrey.

Tests for antibody to MAP were done on all sera by a complement-fixation test as described by Widdowson, Maxted & Pinney (1971).

Indirect bactericidal tests for M antibody were performed as described by Maxted, Widdowson & Fraser (1973). Sera from patients infected with a known M type were tested against a stock strain of that M type or the patient's own strain. A heterologous type was included in each test as a control.

Anti-opacity factor tests were performed on sera from patients infected with an OF positive serotype as described by Maxted *et al.* (1973).

RESULTS

Antibody responses in eleven patients colonised with Group A streptococci (see Fig. 1).

The ASO response was moderate, or poor, except in three of the four patients with type T12/M12 infections, who showed increases in titre from < 50 to > 800 units. The fourth patient infected with streptococci of this type (no. 10) had only a small burn (2%) that was colonized with streptococci of type T12/M12 from the 8th to the 25th day and showed no ASO response.

The anti-DNAase B response was much more vigorous than the ASO response in most of the patients. Thus patients nos. 1, 2, 3, 4, 6 and 7 showed very rapid rises in anti-DNAase B titre to levels of > 9600 and patients nos. 8 and 11 also showed increases to > 2500.

In patient no. 2 the high titre was sustained but in patient no. 1 it was only

Table 1. *Burned patients colonized with streptococci*

Patient no.	Age (years)	Sex	Percentage of body surface burned	Infecting streptococci			Duration of infection (days)*	Day* of collection of		Number of sera tested
				Group	T	M		1st serum	last serum	
1	23	F	50	A	6	6	2 only	2	51	7
2	19	M	46	A	6	6	5–33	13	69	4
3	30	M	30	A	11	78	1–9	1	23	8
4	21	M	40	A	12	22	7–14	1	40	4
5	14	M	7	A	12	22	21–31	2	43	5
6	33	F	18	A	UT	UT	3 only	2	44	7
7	56	F	13	A	12	12	6–31	3	71	10
8	77	F	5	A	12	12	2–34	1	64	10
9	28	M	11	A	12	12	22–46	2	254	9
10	24	F	2	A	12	12	8–25	2	57	5
11	17	F	16	A	6	6	3–13	2	16	3
12	12	M	28	G	NT	NT	2–12	5	48	4
13	18	M	25	G	NT	NT	7 only	1	24	4
14	25	M	50	C	NT	NT	3–10	3	53	4

* Day 1 – day of burn.
UT = Untypable NT = not tested.

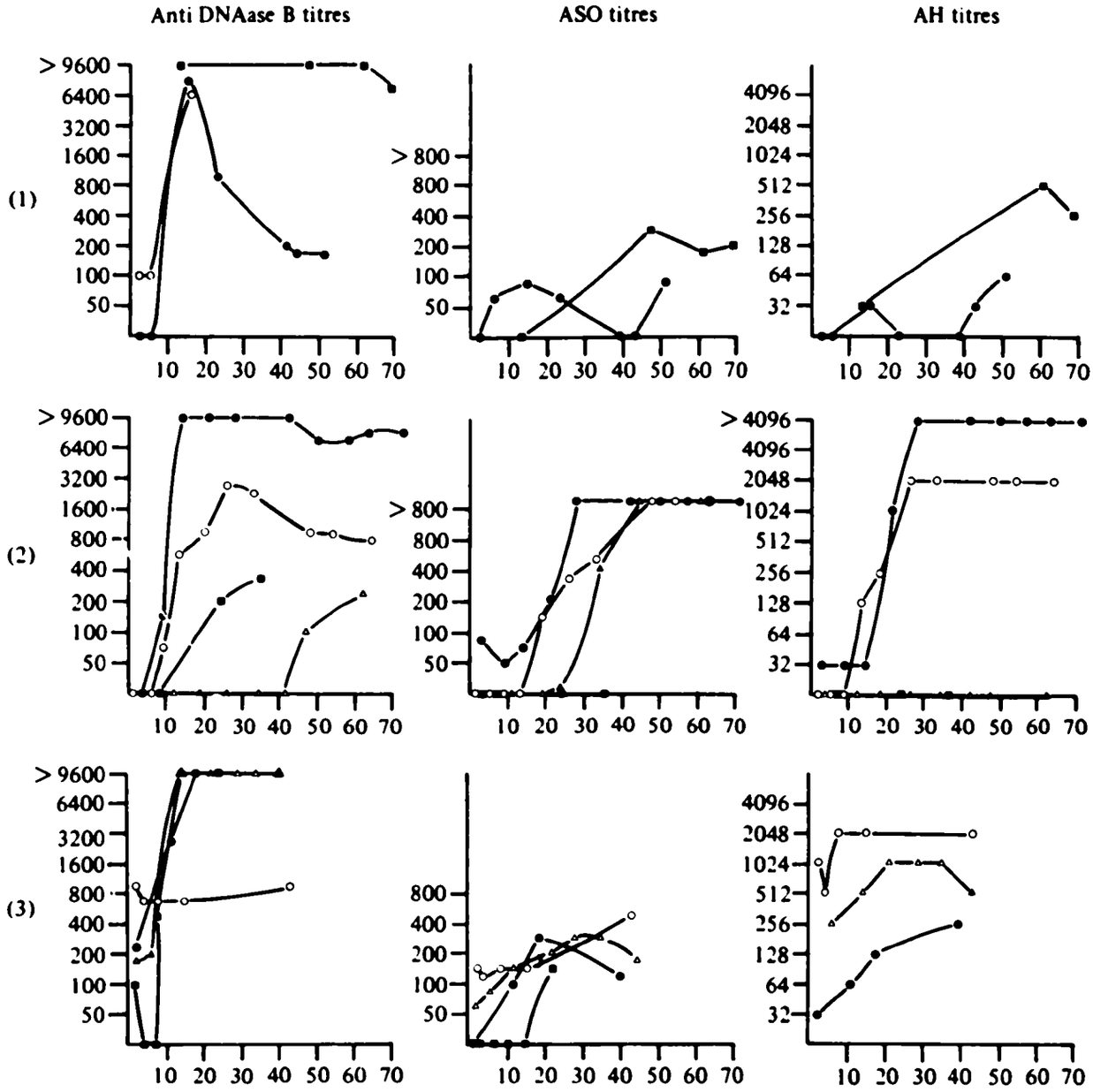

Fig. 1. Streptococcal antibody titres in eleven patients with burns colonized by Group A streptococci. 1 = Patients colonized with type T6/M6: ●, patient no. 1: ■, patient no. 2; ○, patient no. 11. 2 = patients colonized with type T12/M12: ●, patient no. 7; ○, patient no. 8; △, patient no. 9; ■, patient no. 10. 3 = Patients colonized with type T12/M22: ●, patient no. 4; ○, patient no. 5. Patient colonized with type T11/M78: ■, patient no. 3. Patient colonized with untyped strain: △, patient no. 6.

transient. The infecting streptococcus was isolated from patient no. 1 only on the second day, though the burn was swabbed on 15 subsequent days. However, this patient also failed to produce antibody to staphylococcal α haemolysin and micrococcal nuclease despite prolonged colonization of the burn with *Staph. aureus* and staphylococcal bacteraemia on days 40–42. Patient no. 2, however, whose burn was also colonized with *Staph. aureus*, developed a high titre of both antibodies.

The anti-hyaluronidase response. The most significant AH rises were in patients colonized with streptococci of type T12/M12. Two of these (nos. 7 and 8) showed strong AH responses and one (no. 9) showed a small rise comparable with his moderate anti-DNAase B titre, but this patient was not colonized with streptococci

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Table 2. *Antibody response to streptococcal cellular antigens in patients with burn injuries infected with group A streptococci*

Patient number	Infecting serotype		Antibody to M-associated protein				Type-specific 'bactericidal' M antibody		Antibody to type-specific opacity factor
			Increase in titre			Time* (days) to reach maximum titre	Response	Time (days)* to become positive	
	M	T	Response	From	To				
1	6	6	−	...	...	...	+ (transient)	3	... †
2	6	6	−	...	...	...	+ (transient)	8	... †
3	11	78	+	< 10	20–40	22	NT	...	+ (at 2 days)
4	12	22	−	...	...	...	+	11	−
5	12	22	−	...	...	...	−	...	−
6	UT	UT	+	< 10	20–40	33	NT	...	NT
7	12	12	+	20–40	> 320	22	+	44	... †
8	12	12	+	< 10	320	31	+	46	... †
9	12	12	+	< 10	80–160	40	+	40	... †
10	12	12	−	...	...	...	−	...	... †

* Time from first day of colonization by streptococci.
† opacity factor negative serotypes.
... = not applicable UT = Untypable NT = Not tested.

until the 23rd day. The fourth patient (no. 10) had a very small burn and no rise at all in AH titre. Patient no. 1 who gave only a transient ASO response, failed to produce significant amounts of AH.

The anti-MAP responses were negative or poor (< one two-fold dilution) in all patients (Table 2) except for three of the four patients with T12/M12 infections, two of whom showed rises in anti-MAP titre from < 10 to ⩾ 320.

Type-specific antibodies (*Table* 2). Patients nos. 1 and 2, who both suffered from severe burns colonized with type T6/M6, developed 'bactericidal' antibody against the homologous type only a few days after infection. This antibody was transient in both patients and in patient no. 1 was very weak. In patient no. 3, who developed streptococcal bacteraemia from the third to the fifth day, bactericidal antibody was not tested for, but from the second day of colonization onwards this patient's serum had low levels (titre < 1/5) of antibody to the type-specific opacity factor of M-type 78. Patients nos. 4 and 5 were both infected with streptococci of type T12/M22 but only one (no. 4) developed type-specific 'bactericidal' antibody. However, this patient had an extensive burn (40 %) and was infected on day 7, but patient no. 5 had a smaller burn (7 %) and was not shown to be colonized with streptococci until the 21st day. Neither of these patients developed anti-OF antibody to M-type 22.

In contrast to patients nos. 1 to 4, who developed type-specific antibody (either to OF or M antigen) within ten days of colonization with a streptococcus, patient nos. 7 to 9 took between 38 and 46 days to develop bactericidal antibody to their infecting strain (type T12/M12). All three were suffering from smaller burns (5–13 %) than patients nos. 1 to 4 (30–50 %).

Antibody responses to infections with streptococci of groups C and G

Two patients (nos. 12 and 13) whose burns were colonized with group G streptococci showed significant rises in anti-DNAase B titre, one of them (no. 12) from 200 to > 9600 and one (no. 13) also had a rise in ASO titre from 355 to > 800. A patient infected with a group C strain showed a small rise in ASO titre (< 50 to 155) but a steep increase in anti-DNAase B titre (< 50 to 3200). None of these patients showed a significant anti-MAP response and type-specific antibodies were not tested for.

Antibody responses in patients not infected with streptococci

Of 19 patients who had no bacteriological evidence of streptococcal infection only three showed significant increases in titre of one or more streptococcal antibodies during the sampling period, presumably due to undetected infection. Of the remaining 16 patients three had no detectable streptococcal antibodies and 13 had low titres of most of the antibodies on admission. All of them showed slight falls or stationary titres throughout their stay in hospital. The number of sera from each patient ranged from four to 10.

DISCUSSION

After infection with a group A streptococcus, antibodies to a wide variety of extracellular and somatic antigens can be detected in the patient's serum. The duration and magnitude of these antibody responses may depend on the serotype of the infecting strain, the site of infection and whether the antibody is directed against a cellular or extracellular component of the streptococcus. Thus the anti-MAP and type-specific antibody responses after throat infection with opacity-factor positive M types is usually poor compared with opacity-factor negative types, presumably due to the poor antigenicity of some M proteins (Widdowson *et al.* 1974). The type-specific antibody response after skin infection seems to be rather poor and occurs infrequently (Widdowson *et al.* 1974; Potter *et al.* 1971; Dillon *et al.* 1979). Patients with streptococcal pyoderma have considerably lower titres of ASO than do patients with streptococcal sore throat, but titres of anti-DNAase B are often very high in cases of skin infection (Anthony, Perlman & Wannamaker, 1967; Dillon & Reeves, 1969). In general, the extracellular products of the group A streptococcus elicit a rapid antibody response that reaches a maximum 2–3 weeks after infection and then declines over the next 4–6 months, but antibodies to the cellular antigens are slower to appear (taking up to 6 weeks) and may persist in the serum for many years after infection (Lancefield, 1959; Dudding & Ayoub, 1968).

The anti-DNAase B response in most of the colonized burns patients was both rapid and vigorous except where the burns were very small (e.g. patient no. 10) or if colonization occurred very late (e.g. patients nos. 5 and 9). It appears that a fresh burn colonized by streptococci allows rapid absorption of large quantities of DNAase B; the response is thus very similar to that observed in streptococcal impetigo. Colonization of a healing burn, even if it is profuse, does not appear to stimulate anti-DNAase B production.

The ASO responses of most of the patients were poor; this is in agreement with observations on patients with streptococcal impetigo, in whom, it is believed that the antigenicity of streptolysin O is depressed by combination with free cholesterol in the skin lesions (Kaplan & Wannamaker, 1976). However, infection of burns with T12/M12 even when the burns were small (patient no. 8) or colonization occurred late (patient no. 9) gave a high ASO response though this was rather slow to develop. None of the patients colonized with M-type 12 streptococci showed clinical evidence of throat infection or complained of sore throat while in hospital. All had negative nasal and throat swabs on admission but unfortunately swabs were not taken during streptococcal colonization of their burns. Thus the possibility that the three patients with a high ASO response had developed sub-clinical throat infections cannot be excluded.

Three of the patients infected with T12/M12 (nos. 7, 8 and 9) developed antibody to the cellular antigens MAP and M protein. The type-specific antibody was slow to develop and the dynamics of both responses were similar to that observed after throat infection with an OF-negative serotype. On the other hand, the antibody response to cellular antigens in the patients colonized with other serotypes

(T6/M6, T12/M22 and T11/M78) was generally poor. Thus none of these patients responded to MAP antigen and the M-antibody responses were atypical in that antibody developed very quickly (3–10 days after infection), and in the case of the type 6 infections was very transient. There was insufficient serum to demonstrate if the bactericidal antibodies were of the IgG or the IgM class. The magnitude of the anti-MAP response is known to be dependent on the serotype of the infecting strain and OF-positive serotypes (e.g. M22) are known to be poorly antigenic, and even among the OF-negative types there is great variation in ability to stimulate anti-MAP (Widdowson *et al.* 1974).

The influence of antibiotic treatment on the ASO and typespecific antibody response after throat infection has been studied (Denny, Perry & Wannamaker, 1957); penicillin therapy given early enough greatly reduces the magnitude of the response. It is difficult to assess the effect of chemotherapy on the antibody responses of these burned patients, but in each case streptococcal surface growth was profuse before treatment was started and it is unlikely to have had much effect in most cases. Moreover, three patients who had M-type 12 infections were given prolonged treatment with phenoxymethyl penicillin and in spite of this developed type-specific antibody and had high ASO titres.

These studies show that, even in patients with severe burns, the antibody response to some streptococcal antigens, for example, DNAase B, is usually vigorous, though suggestive evidence of toxic immunosuppression was found occasionally (e.g. in patient no. 1). However, responses to cellular antigens were often weak and transient. Although some serotypes induced 'bactericidal' antibody in burned patients very much earlier than is usual in throat infection, it is unlikely that these play any significant role in limiting the infection.

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