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Serological typing of coagulase-negative staphylococci using monoclonal antibodies

C. GABELISH[1], C. HARBOUR[1], M. A. BEARD-PEGLER[1], E. STUBBS[1], R. STEFFE[1], M. LARGE[2], A. VICKERY[2] AND R. BENN[2]

[1] *Department of Infectious Diseases, University of Sydney, NSW 2006, Australia*
[2] *Department of Microbiology, Royal Prince Alfred Hospital, Sydney, NSW 2050, Australia*

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SUMMARY

This investigation was to determine whether monoclonal antibodies (Mabs) could be used to differentiate coagulase-negative staphylococci (C-NS) at species and strain level. Mabs were produced to four *Staphylococcus epidermidis* strains, two *S. haemolyticus* strains, one *S. saprophyticus* strain and one *S. warneri* strain. A panel of nine antibodies was tested for species and strain specificity against five type strains and 65 clinical isolates of C-NS by enzyme-linked immunosorbent assay (ELISA). Species specificity was found with Mab D150 produced to one *S. haemolyticus* strain. Using Mab D150 and Mab D198 in conjunction, identification of 90% of *S. haemolyticus* isolates to species level was achieved. *S. saprophyticus* Mab K84 reacted with most other strains of C-NS tested but only three *S. haemolyticus* strains (16%). This finding provides further evidence that *S. haemolyticus* possesses different surface determinants to other C-NS which could form the basis of a typing scheme for *S. haemolyticus* using Mabs D150, D198 and K84.

INTRODUCTION

Coagulase-negative staphylococci (C-NS) cause serious nosocomial infections [1–3]; however, to obtain a positive identification of species or strains is both cumbersome and problematic because at present a combination of typing methods must be used. There is a need for accurate identification of individual isolates of C-NS as they are ubiquitous skin flora and the most frequent contaminants of clinical specimens which makes the determination of clinical significance difficult. Immunological studies [4] of C-NS using polyclonal antisera to type strains, despite the cross-reactivity seen, suggested that species specific agglutinogens existed on the surface of these organisms. The potential for developing a serological typing scheme was apparent in preliminary work [5]. Therefore, the purpose of the current work was to investigate further this potential for serologically typing different C-NS species by using monoclonal antibodies (Mabs) generated by hybridoma technology. Mabs were produced to 8 strains of 4 species

Correspondence: Dr C. Harbour, Department of Infectious Diseases, University of Sydney, NSW 2006, Australia.

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of C-NS and a panel of 9 of the Mabs was assessed for strain and species specificity against 70 strains of C-NS.

MATERIALS AND METHODS

Bacterial strains

Isolates of C-NS were obtained from routine specimens collected at the Royal Prince Alfred Hospital, Sydney, and reference C-NS type strains were obtained from the American Type Culture Collection. Clinical strains were classified to species level using API 20 Staph kits (API System, Montalieu Vercieu, France) as specified by the manufacturer.

Eight strains, designated prototype strains, were used to immunize mice for the production of Mabs: *Staphylococcus haemolyticus* strains D150 and D198 were isolated from the peritoneal dialysis fluid (PDF) of continuous ambulatory peritoneal dialysis (CAPD) patients with episodes of peritonitis; *S. epidermidis* strains SE10 and SE360 were kindly provided by Professor Ohshima [6]; *S. epidermidis* strain SE45 was isolated from a patient with a cerebrospinal fluid shunt infection; *S. epidermidis* type strain ATCC14990; *S. warneri* strain D48 and *S. saprophyticus* strain K84 were isolated from CAPD patients with episodes of peritonitis (Table 1).

The 70 C-NS strains tested with the Mab panel included five type strains, *S. haemolyticus* (ATCC29970), *S. hominis* (ATCC27844), *S. epidermidis* (ATCC14990), *S. warneri* (ATCC27836) and *S. saprophyticus* (ATCC15305); 62 clinical isolates from the skin and PDF of CAPD patients included 19 *S. haemolyticus* strains, 14 *S. hominis* strains, 23 *S. epidermidis* strains and 6 *S. warneri* strains; and the 3 *S. saprophyticus* isolates were 2 from urine cultures and 1 from PDF.

All strains were stored at −20 °C in skim milk/glycerol storage medium. Cultures were prepared by incubation in Tryptic Soy Broth for 20 h at 37 °C, washed and resuspended in saline. Prototype strains for Mab production were formalin (0·5 %) killed overnight and one prototype strain, SE360, was also heat killed by boiling for 30 min. Strains used for assessing Mab specificity were formalin killed.

Monoclonal antibodies

Bacterial vaccines, 0·3 ml of 6×10^9 organisms/ml (heat killed or formalin killed), were administered intraperitoneally, with the final immunization given 3 days before fusion. Spleen cells derived from immune mice were fused with mouse myeloma fusion partner, P3-NSI/1-g4-1 in 60 % polyethyleneglycol 4000 at a ratio of 10:1 lymphocyte:myeloma [7]. The fusion mixture was plated in 4×96 well microtitre plates. Hybridomas were selected in medium containing hypoxanthine-aminopterin-thymidine and were assayed for antibody production by enzyme-linked immunosorbent assay (ELISA).

ELISA

Assays of cell culture supernatants for antibody production and reactivity of antibodies with C-NS strains were performed by standard ELISA procedures. Strains were used to coat plates (NUNC-Immuno plates F96 with certificate) at a

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Table 1. *Prototype strains used for immunization of mice for hybridoma production*

Species	Strain	Source	Treatment prior to immunization
S. haemolyticus	D150	PDF*	Formalin
	D198	PDF	Formalin
S. epidermidis	SE10	Ohshima et al.	Formalin
	SE360F	Ohshima et al.	Formalin
	SE360B	Ohshima et al.	boiled
	SE45	CSF†	Formalin
	ATCC 14990	Type strain	Formalin
S. warneri	D48	PDF	Formalin
S. saprophyticus	K84	PDF	Formalin

* PDF, peritoneal dialysis fluid.
† CSF, cerebrospinal spinal fluid.

cell density of 1×10^9 organisms/ml overnight at room temperature. Bound antibodies were detected using horse-radish peroxidase-labelled goat anti-mouse IgA+G+M(KPL/CSL Melbourne) and optical densities were recorded on a plate reader (Flow Laboratories Australasia Pty. Ltd) at 414 nm at 10, 20 and 30 min time intervals. Positive and negative controls were included in each assay.

RESULTS

A panel of nine Mabs was tested against different C-NS strains and the results are presented in Tables 2–4. Initially the Mabs were tested against the nine prototype C-NS strains used to generate the Mabs and these results are shown in Table 2. These results indicated that Mab D150 and Mab D198, produced to *S. haemolyticus* strains D150 and D198 respectively, were species specific but recognized different antigens since they reacted with different strains of *S. haemolyticus*. In addition the Mab raised to *S. epidermidis* strain SE10 appeared to be unusual in that it reacted with only SE10 and no other prototype strains, whereas the other four Mabs generated using *S. epidermidis* strains reacted with all prototype strains except the *S. haemolyticus* strain D150 and the *S. saprophyticus* strain K84.

These results prompted further screening tests to determine whether or not the Mabs could be used to differentiate both C-NS type strains (Table 3) and clinical isolates (Table 4).

The results presented in Table 3 confirm both the different specificities of Mab D150 and Mab D198, since only the latter reacted with the *S. haemolyticus* type strain, and the species specificity of these Mabs since they did not react with any other type strain. The results shown in Table 4 confirm the species specificity of Mab D150 but not Mab D198 which reacted with 4 of the 14 strains of *S. hominis* tested. Neither of these antibodies reacted with any of the 23 *S. epidermidis* isolates tested. In contrast, the Mabs raised to the *S. epidermidis* strains SE360, SE45, ATCC14990, reacted with the majority of isolates tested. The reaction profile of Mab SE10, produced to strain SE10, was different to the other *S.*

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Table 2. *ELISA reactions* of prototype strains and the monoclonal panel*

Prototype strains	Monoclonals† D150	D198	SE10	SE360F‡	SE360B§	SE45	ATCC 14990	D48	K84
D150	+	–	–	–	–	–	–	–	–
D198	–	+	–	+	+	+	+	–	–
SE10	–	–	+	+	+	+	+	+	–
SE360F	–	–	–	+	+	+	+	+	+
SE360B	–	–	–	+	+	+	+	+	–
SE45	–	–	–	+	+	+	+	+	+
ATCC 14990	–	–	–	+	+	+	+	+	+
D48	–	–	–	+	+	+	+	+	+
D84	–	–	–	–	–	–	–	–	+

* Positive (+) and negative (–) reactions. † Species to which Mabs were raised: *S. haemolyticus*, D150, D198; *S. epidermidis*, SE10, SE360F, SE360B, SE45, ATCC 14990; *S. warneri*, D48; *S. saprophyticus*, K84. ‡ F, formalin treated. § B, boiled.

Table 3. *ELISA reactions* of coagulase-negative staphylococcal type strains and the monoclonal panel*

Type strain (type number)	Monoclonals† D150	D198	SE10	SE360F‡	SE360B§	SE45	ATCC 14990	D48	K84
S. haemolyticus (ATCC 29970)	–	+	–	+	–	+	+	+	–
S. hominis (ATCC 27844)	–	–	–	+	+	+	+	+	+
S. epidermidis (ATCC 14990)	–	–	–	+	+	+	+	+	+
S. warneri (ATCC 27836)	–	–	–	+	+	+	+	+	+
S. saprophyticus (ATCC 15305)	–	–	–	–	–	+	+	+	+

* Positive (+) and negative (–) reactions. † Species to which Mabs were raised: *S. haemolyticus*, D150, D198; *S. epidermidis*, SE10, SE360F, SE360B, SE45, ATCC 14990; *S. warneri*, D48; *S. saprophyticus*, K84. ‡ F, formalin treated. § B, boiled.

epidermidis Mabs reacting with only a minority of the isolates tested. Mab D48, generated using a *S. warneri* strain, produced a similar reaction profile to Mabs SE360, SE45 and ATCC14990.

As stated in the Methods, Mab SE360F and Mab SE360B were produced using formalin-treated and boiled preparations of *S. epidermidis* strain SE360 respectively. The reactions of these antibodies were similar with the exception that Mab SE360F reacted with the *S. haemolyticus* type strain and with one more strain of *S. saprophyticus*.

Mab K84, produced to a *S. saprophyticus* strain, reacted with the type strain but did not react with the three clinical isolates of *S. saprophyticus* tested. Although it typed 100% of the clinical *S. epidermidis* strains it did not react with all the prototype strains. It did react with 13 of the 14 *S. hominis* strains but with only 3 of the 19 *S. haemolyticus* strains tested.

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Table 4. *Percentage of positive ELISA reactions shown by clinical isolates and the monoclonal panel*

Clinical strain (number)	Monoclonals*								
	D150	D198	SE10	SE360F†	SE360B‡	SE45	ATCC 14990	D48	K84
S. haemolyticus (19)	74	16	21	84	84	68	63	63	16
S. hominis (14)	0	20	21	93	93	100	100	100	93
S. epidermidis (23)	0	0	9	100	100	100	100	100	100
S. warneri (6)	0	0	0	83	83	100	100	100	17
S. saprophyticus (3)	0	0	33	67	33	67	67	33	0

* Species to which Mabs were raised: *S. haemolyticus*, D150, D198; *S. epidermidis*, SE10, SE360F, SE360B, SE45, ATCC 14990; *S. warneri*, D48; *S. saprophyticus*, K84. † F, formalin treated. ‡ B, boiled.

DISCUSSION

In order to associate a specific infection in a patient with a particular C-NS isolate it is essential to use an accurate identification scheme since C-NS are ubiquitous skin flora and the most frequent contaminants of clinical specimens. Unfortunately a widely acceptable single successful typing scheme to identify both species and strains of C-NS does not exist. In other work [3] we have attempted with some success to use biotyping, phage typing and plasmid profile analysis for an identification strategy for C-NS. In this current work our aim was to produce Mabs to species-specific antigens of C-NS and to investigate the possibility of producing strain specific Mabs within the species *S. haemolyticus* and *S. epidermidis*. The theoretical basis for this work was provided by Pillet and Orta [4,8] who, using polyclonal antisera raised in rabbits, reported an association between C-NS species and serotype.

In our work we have demonstrated species specificity for *S. haemolyticus* using Mab D150. In addition, when this antibody was used in conjunction with Mab D198, 90% of *S. haemolyticus* strains could be identified. It would appear that, since these antibodies react with different strains of *S. haemolyticus*, they are directed to distinct antigenic sites on the cell surface. The site recognized by Mab D150 was not found on any of the other 70 C-NS strains tested but the Mab D198 site was detected on 4 out of 14 *S. hominis* strains tested. Thus it is possible that these antibodies could form the basis of an identification scheme for *S. haemolyticus*. This would be extremely useful since these organisms are the second most common cause of C-NS infections and are frequently resistant to many antibiotics [9]. The differences we have shown between the reactions of the *S. haemolyticus* strains with the Mab panel compared to the reactions of the *S. epidermidis* strains are indicative of distinct cell wall structures and may reflect other differences in the biology of these organisms, e.g. the *S. haemolyticus* strains are highly resistant to antibiotics and do not carry many plasmids (unpublished

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observation). In addition we have shown that the Mabs, D150 and D198, could be recognizing two distinct groups within the *S. haemolyticus* species and further work is under way to test this hypothesis. The reactions obtained with the Mabs, particularly Mab D198, suggest that the group of *S. haemolyticus* strains recognized by this Mab are more closely related to *S. hominis* than *S. epidermidis* thus supporting the DNA homology and comparative immunological studies of Schleifer and colleagues [10] who reported a significantly closer relationship between *S. haemolyticus* and *S. hominis* than to either *S. epidermidis* or *S. warneri*. It is difficult to discuss the significance of the reactions shown by Mab K84, produced to *S. saprophyticus*, since so few strains were tested but it is clear that the antigen to which Mab K84 binds is not present on all strains of this species but recognized all *S. epidermidis* strains tested and only 16% of *S. haemolyticus* strains. This antibody could therefore be used along with Mab D150 and Mab D198 to differentiate *S. haemolyticus* from *S. epidermidis* strains.

The antibodies produced to 3 of the 4 *S. epidermidis* strains, i.e. SE360, SE45 and ATCC14990, recognized antigens present on the majority of C-NS strains tested. In contrast Mab SE10 gave a completely different reaction profile suggesting that the organism, i.e. strain SE10, to which the Mab was made, was an unusual *S. epidermidis*. We therefore carried out further biochemical testing and discovered that in fact this strain was more closely related to *S. xylosus* group than the *S. epidermidis* group. This finding, in an indirect way, confirms in our opinion the potential of using Mabs for distinguishing between C-NS species.

In summary, we have demonstrated the successful production of species specific Mabs to C-NS thus confirming Pillet and Orta's [4] original assertion that C-NS species could be differentiated serologically. Work is now in progress to verify these findings using a larger number of C-NS and to attempt to identify the distinctive antigen recognized by Mab D150 on the majority of *S. haemolyticus* strains.

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