VI. Detection of Implanted Staphylococcus Aureus Strain

Use of Serological and Phage Typing

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A recent approach to the control of staphylococcal problems in hospital nurseries involves the implantation of a penicillin-sensitive staphylococcus (the 502A strain) in infants in an effort to interfere with colonization by more virulent organisms.[1,2] The evaluation of the results of such implantation experiments depends upon accurate and definitive identification of the implanted strain and the ability to differentiate it from other strains of coagulase-positive staphylococci found in the environment. The use of two or more distinct typing systems, especially for cultures from lesions and other infections, greatly improves the reliability of the experiment. Toward this end selected representative staphylococcus strains from the Georgia study [2] were referred to the research laboratory for special serological and phage studies.

Materials and Methods

The serological system used in this study was developed by Oeding [3-5] and by Haukenes and Oeding.[6] Methods of producing reagents and performing the tests are described in a recent paper by Cohen and Oeding.[7] The fluorescent antibody method was used to type several auto-agglutinating strains. Antisera for factors, *a*, *b*, c_p (polyvalent), c_1, *h*, *i*, *k*, and *m* were used. (During the course of this study the monovalent reagent for factor c_1 was developed by adsorption of F21 antiserum with strains 1503, 2095, and Wood 46.) Several isolates from the stock 502A strain, a selected group of nasal and umbilical cultures from inoculated and uninoculated babies, and a few cultures from lesions were typed by both phage and serological procedures. One hundred colonies of the 502A strain were phage typed to determine the range of variation in vitro. The four colonies listed in Table 1 represent the extent of variation observed.

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Results

The 502A strain reacted strongly with the monovalent c_1 antiserum, the polyvalent c_p antiserum, and with the divalent *ci* antiserum. In addition, it reacted weakly with polyvalent *b* antiserum. The strain was therefore designated type (*b*)c_1 but the strong c_1 reaction was the most useful in identifying it. The serotypes and phage patterns of representative strains from a nursery study are given in Table 1. Strains of the 52/52A/80/81 complex, strains of other phage patterns, and a number of strains not typed by our phages exhibited a variety of serological types. In general, those strains associated with each other by phage type were also associated with each other by serotype.

In Table 2 the serology and phage results are summarized according to epidemiological data on sources of the cultures. Twenty-three strains with serology characteristic of the 502A staphylococcus (ie, (b) c_1 or [c_1]) were identified among the cultures referred for special study. Twenty of these were from inoculated babies, including three from a parallel study in Cincinnati which were tested in order to ascertain the similarity between

TABLE 1.—*Comparison of Serological Type and Phage Type of Some Representative Strains From a Nursery Study of Implantation With the 502A Strain*

No. Tested	Source	Serotype	Phage Patterns
4	502A	(b) c_1 or c_1	6/7 (42E/47/53/83A/81) *; 6/7/47/53 (42E/54/77/81); 7/53/ (6/42E/47/54/77/42D/81);6/7/47/53/83A (42E/54/77/81)
16	Inoculated babies	(b) c_1 or c_1	6/7 (42E/47/53/83A/81) and similar patterns
4	Inoculated babies	(b) c_1 or c_1	7; 7 (53); or 7 (53/83A)
1	Uninoculated baby	(b) c_1	6/7/53 (42E/47/54/81/83A)
1	" "	c_1	7 (6/47)
1	" "	bcim	3A (3B/3C)
6	" babies	abc_p	29/52/52A/79/6/7/47/53/54/75/83A/81 (80/42E)
1	Breast abscess of mother †	h	No reaction
2	Uninoculated babies	h	No reaction
1	Uninoculated baby	abc_pk	52/52A/80/81
1	" "	abc_phk	52/52A/80/81
1	" "	abc_1	75/77 (47)
2	" babies	k	No reaction
1	" baby	No reaction	No reaction

* Numbers in parentheses represent weak reactions.
† Culture taken 6 weeks after discharge from the hospital.

the 502A progeny in the separate studies. Eighteen of the (b)c_1 or c_1 strains were considered to be the same as the 502A strain by phage typing, although interpretation of the phage results was difficult, as mentioned previously, because of variations in intensity of reactions. Five (b)c_1 or c_1 strains (four from inoculated babies) had phage patterns which were distinctly different from that of known 502A cultures. One culture from an uninoculated baby was indistinguishable from the 502A strain by serology and phage type. This appears to represent cross-colonization with the 502A strain. One culture from an inoculated baby was distinct from the 502A strain by both procedures. This culture was isolated from the same plate as a culture with both serotype and phage type characteristic of the 502A strain. All of the 27 strains differentiated from the 502A strain by serology were also differentiated from it by phage typing.

Comment

In order to evaluate the effect of nasal inoculation of the 502A staphylococcus strain in newborn infants an intensive culturing and phage typing program was carried out by the CDC Epidemiology Branch Laboratory. Positive identification of the 502A strain proved to be difficult because of variability of the phage patterns. Therefore, selected cultures were referred to the Staphylococcus and Streptococcus Unit for confirmatory phage typing and serological studies. In both laboratories the variations in phage reactions or the complete absence of susceptibility to the basic set of phages resulted

TABLE 2.—*Comparison of Serological and Phage Differentiation of Fifty Selected Staphylococcus Strains From a Nursery Study of Implantation With the 502A Strain*

	Serotype					
	(b) c_1 or c_1 (Characteristic of the 502A Strain)			Distinct from (b) c_1 or c_1		
Source	Total	Like 502A by Phage Typing	Unlike 502A by Phage Typing	Total	Like 502A by Phage Typing	Unlike 502A by Phage Typing
Inoculated babies	20	16	4	1	0	1
Uninoculated babies and other sources	3	2	1	26	0	26
Total	23	18	5	27	0	27

in rather poor definitive identification of many of the strains under study.

In contrast, all but one of the 50 strains were typed successfully by serological procedures; 46 by agglutination, and 3, which agglutinated spontaneously, by a fluorescent antibody method. The strong c_1 antigen in the 502A strain provided a ready marker to its identification, especially in view of the apparent absence of similar strains in the nursery environment. Five $(b)c_1$ or c_1 strains which had phage patterns distinctly different from the majority of 502A cultures were more difficult to classify. However, in view of the marked variability in phage reactions of known 502A subcultures (Table 1) one is inclined to consider the serological reactions as the more reliable in this study.

Studies of strains by both phage typing and serology by Oeding and Vogelsang,[8] Oeding and Williams,[9] and our own laboratory (unpublished data) indicate that the serological type and phage type are based on different markers. Where positive identification of a culture is essential in the evaluation of important experiments, the use of both systems offers an obvious advantage over either system alone.

In the study presented here, serological typing was useful in several instances in making judgments as to the successful colonization with the 502A strain and its ability to interfere with colonization by other strains. Serology also indicated that several lesions of questionable etiology were due to organisms other than the 502A strain.

Summary

During a study of the effect of artificially introducing a penicillin-sensitive strain (502A) into a nursery in an effort to limit the dissemination of more virulent staphylococci, representative cultures were selected for special serological and phage studies. The 502A strain was found to possess a strong c_1 antigen by which it could be distinguished from other strains in the nursery environment. Serological typing proved valuable in assessing the results of the experiments.

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