Further Studies on Antigen Variation in
Staphylococcus aureus

ARTUR T. PEREIRA
Instituto Bacteriológico Câmara Pestana, Faculty of Medicine, University of Lisbon, 1100 Lisbon, Portugal

The plating of successive Staphylococcus aureus subcultures of daily transfers proved that discontinuous variation resembling a genetic mutation and selective outgrowth of the variant are responsible for antigen variation. Every subculture of S. aureus, when repeatedly transferred, contained a mixture of cells with original antigen 17 (or 13) and final antigen 1 (or 3) that are relevant for research, serological diagnosis, and epidemiological study of staphylococcal diseases.

Antigen loss variation in Staphylococcus aureus was first reported in 1961 by Torres Pereira (12), who found that most recently isolated strains possessed one of two surface antigens, designated 13 and 17, which were detected by agglutination with absorbed sera. On serial subculture each strain lost its respective antigen 13 or 17 after 10 to 50 transfers. Each antigen was replaced by another; antigen 13 was replaced by 3, and antigen 17 was replaced by 1. The phage typing pattern of the strains remained constant, but virulence for mice challenged by intracerebral injections was reduced after the variation (14, 15). The phage typing patterns suggested that the strains of Cowan (6) serotypes I and III originally possessed antigens 17 and 13, respectively, but not antigens 1 and 3 (13).

The phenomenon of antigen variation must be taken into account in any scheme for serotyping S. aureus. If sera are produced to cultures in which antigen 13 or 17 is partly replaced by antigen 3 or 1, the results may suggest the presence of a variety of antigens and types not detected in freshly isolated strains. It is thought that the variety of factors identified by Oeding (9) may be partly the consequence of antigen variation in the strains he used to produce typing sera.

The mechanism of the variation has not been elucidated. Observations suggesting that the variation is due to the selective outgrowth of a variant, probably mutant bacteria, are described in this paper.

MATERIALS AND METHODS

Strains. Several recently isolated strains from hospital patients were studied: 9530 (antigen 13*) from a blood culture; 5196 (antigen 13*) from a wound; 82893 (antigen 13*) from pus; 4681 (antigen 13*) from a wound; 5606 (antigen 13*) from a patient with osteomyelitis; 5270 (antigen 17*) from pus; 5312 (antigen 17*) from pus; 381 (antigen 17*) from a blood culture; 4178 (antigen 17*) from pus; and 5822 (antigen 17*) from pus. They were all S. aureus coagulase and deoxyribonuclease positive, with different phage typing patterns. Cultures were grown on Columbia agar base (Oxoid Ltd.) for 18 h at 37°C. Standard strains 33 and 7 were used for the absorptions.

Slide agglutination. Slide agglutinations were done with absorbed sera made as described previously (11, 12).

Briefly, agglutinating antisera against antigens 17 and 13 were obtained from rabbits injected with bacteria from overnight nutrient agar slant cultures of recently isolated strains. The staphylococci were killed with 0.5% Formalin. Animals were injected for 3 successive days for the first 2 weeks and with living cultures for 3 successive days during week 3. The original strains were kept at 4°C for at least 1 month.

Agglutinating antisera against antigens 1 and 3 were obtained from rabbits injected with strains after many transfers which manifested the variation.

Sera diluted 1:5 with 0.9% NaCl were absorbed with live standard strain 33, obtained from agar plates after 18 h of incubation at 37°C, for 4 h in a water bath at 50°C. As a rule, two or three absorptions were sufficient. A satisfactory antiserum will agglutinate solely the strain bearing the corresponding antigen.

Absorbing strain 33 was used for preparing antisera 17, 13, and 1. Standard strain 7 which contains neither of the four antigens was used for preparing absorbed antiserum 3.

Slide agglutinations were read in the first 15 s of mixing. Only strong and immediate reactions were recorded as positive.

RESULTS

Ordinary daily subcultures and antigenic variation. All staphylococci under study were transferred daily, and slide agglutination tests for antigenic changes were performed (Fig. 1 and 2).

As previously published, the variation was easily obtained with every strain studied either with antigen 13 (five strains) or 17 (five strains). Although, in selected strains, 150 daily transfers had been done, the change usually appeared...
were absent.

between subcultures 20 and 30.

Antigen variation resulting from selective outgrowth of a genetic variant. To discover whether the antigenic variant 1* (or 3*), manifested after daily subcultures, had any selective advantage over the original strain 17* (or 13*), a number of tubes containing successive subcultures were preserved at 4°C. These slants were subcultured on agar simultaneously. From each agar plate, 30 single colonies were again subcultured and analyzed by slide agglutination the next day.

Colony-forming units with antigen 1* (or 3*) were absent in the first transfers, but were prevalent later after many subcultures.

An example of one of several of these experiments is given in Table 1, using strain 381 isolated from a patient with acute endocarditis.

In this experiment, 100% of the original cells contained antigen 17. After transfer 4, 86% of the staphylococci retained the original antigen. After transfers 6, 8, and 10, 66, 36, and 6 to 10% of the staphylococci, respectively, retained antigen 17.

Successive cloned subcultures. The technique of successive clonal subcultures was aimed at proving whether individual colonies retained their antigenic composition (17 or 13) after many successive subcultures.

<table>
<thead>
<tr>
<th>No. of subcultures</th>
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<td>17+1-</td>
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*Culture freshly isolated from acute endocarditis.

**Total number of colonies, 30.

Individual colonies, generally two or three, were subcultured on individual slants for 6 to 8 h at 37°C, analyzed for antigenic composition to confirm antigenic stability, and transferred to agar plates, still on the same day.

Strains 5270, 5312, and 82893 were used. After 29, 40, and 21 successive subcultures, individual colonies did not manifest antigen variation, displaying their original antigen profiles, namely, 1-17*, 1-17+, and 3-13+, respectively.

DISCUSSION

Successive cloned subcultures without evidence of antigenic variation proved that the observable variation is not the result of a gradual change affecting all the bacteria in the culture. Serial subcultures and antigenic analysis of colonies manifested antigen variation which may be due to a discontinuous variation, resembling a genetic mutation followed by selective outgrowth of the variant.

According to Braun (3), most qualitative changes in antigenic characteristics are either mutational in origin or due to viral conversion. Although specific tests to demonstrate that the variation was due to mutation such as the fluctuation or replica plating tests, were not performed, genetic variation seems a valid explanation for the antigen heterogenicity found.

Antigen variation, probably depending on a discontinuous variation and selective outgrowth of the variant, implies that every subcultured S. aureus strain contains a mixture of cells with original antigen 17 (or 13) and final antigen 1 (or
The relative proportions of both depend largely on the number of transfers performed. This interpretation can clarify most of the unexplained observations on virulence or antigenic variation described by investigators such as Brodie et al. (4), Pillet et al. (10), Beining and Kennedy (2), Gorill (7), Koenig and Melly (8), Adlam et al. (1), and Cohen (5). This interpretation also explains the large and varied number of serological patterns described by Oeding (9). Antigen variation in S. aureus should not be ignored; it is relevant for research and for the diagnosis and epidemiology of staphylococcal diseases.

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**LITERATURE CITED**