Lysogenic Conversion in *Staphylococcus aureus* to Leucocidin Production

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Received for publication 8 May 1972

A *Staphylococcus aureus* strain was successively cured of three prophages. A lysogenic conversion by a group A phage to production of Panton-Valentine leucocidin and by a group F phage to staphylokinase production could be demonstrated.

Bacterial strains carrying a prophage are immune to this phage and may show differences in sensitivity to related and unrelated phages in comparison with the phage-free strain. In addition to changing phage sensitivity, a prophage may add a new character or cause the loss of an existing property in the parent strain. In *Staphylococcus aureus*, lysogenic conversion to loss of a Tween-splitting lipase has been described (6). Other workers have reported a simultaneous lysogenic conversion by group F phages to loss of beta toxin and gain of staphylokinase production (8). In this communication, evidence for lysogenic conversion in a strain of *S. aureus* by a group A phage to an increase of Panton-Valentine (P-V) leucocidin production and by a group F phage to staphylokinase production is presented. Lysogenization of strain 557(8), which is supposed to be phage-free, with the converting phages resulted in comparable lysogenic conversions. P-V leucocidin has a specific cytotoxic action on human leukocytes and macrophages. Definite proof of its contribution to virulence of *S. aureus* has not been furnished.

Induction of phage formation in the lysogenic *S. aureus* V4 isolated from a case of osteomyelitis in man was performed by a 30-min exposure to 0.5 μg of mitomycin C per ml (5). Phages of the serological groups A, B, and F were found in the bacterial lysates. The strain was cured of these phages by successive elimination of the group B, group A, and group F phage by induction with mitomycin C (1 μg/ml) in the presence of specific antiphage sera. The group A and group F phage were also eliminated separately from the strain. Single colonies were tested for loss of immunity by cross-streaking. When strain V4 had been consecutively cured of a group B, a group A, and a group F phages, it appeared to be phage-free. This was deduced from the absence of plaque formation when the supernatant fractions of several further inductions were tested on a number of indicator strains.

The cured variants of strain V4 were lysogenized with pure groups B, A, and F phage propagated on the phage-free strain 557. Strain V4 and the cured and lysogenized variants derived from strain V4 were phage-typed and tested for the production of alpha, beta, and delta toxin (2), P-V leucocidin, staphylokinase (4), and a number of metabolic properties (1). The P-V leucocidin was assayed by an antitoxin-combining method involving determination of the L+ dose per milliliter by microscopy examination of human living leukocytes (3). The antiserum used was standardized with the international reference preparation (7). Table 1 shows that strain V4 (29/+), becomes sensitive to phages 52, 52A, 79, and 80 after the loss of the group B phage; to phages Nobel (N) and 7 after the loss of the group A phage; and to phage 81 after the loss of the group F phage. Lysogenization by either of group A, B, or F phages restored the original patterns of sensitivity. All the lysogens and nonlysogens were identical in production of alpha, beta, and delta toxin and in the investigated metabolic properties, but all strains cured of the group A phage showed a considerable loss of P-V leucocidin production. The capacity to form this toxin was restored after lysogenization with the group A phage (Table 1). The growth rates of the cured strains were identical to that of the parent strain, and decrease of P-V leucocidin production was, therefore, not due to altered growth rate. Confirmation of the relationship between P-V leucocidin production and lysogenization by a group A phage was also obtained from the result of the following experiment. The group A phage and strain CV4[−B,A,F] were incubated for 5
min at 30°C (ratio of phage to bacteria, 50:1) and cooled to 4°C. Surviving bacteria were screened for lysogenicity by cross-streaking, and all lysogenized colonies showed changes in P-V leucocidin production.

Strains lacking the group F phage did not produce staphylokinase (Table 1). Production of beta toxin by strain V4 or any of the cured variants was not observed. The lack by some staphylococcal strains of the capacity to form beta toxin has been demonstrated by Winkler, de Waart, and Grootsen (8).

The nonlysogenic strain S57 (beta toxin-positive and staphylokinase-negative) that is sensitive to all phages of group I and III was lysogenized with the group A and the group F phages derived from strain V4. The variant strains showed the characteristic changes in phage-typing pattern, i.e., a loss of sensitivity for phages N and 7 after lysogenization with the group A phage, and for phage 81 after lysogenization with the group F phage. Strain S57 produced 0.013 L + P-V leucocidin per ml. After lysogenization with the group A phage, 0.20 L +/ml was formed. Lysogenization with the group F phage was accompanied by a gain of staphylokinase and a loss of beta toxin production.

Similar lysogenization experiments with phage N and phage 7 in strains CV4 [−B,A,F] and S57 did not produce an increase in P-V leucocidin production.

From these experiments, it can be concluded that strain V4 is converted to a higher production of P-V leucocidin by a group A phage. A second lysogenic conversion to staphylokinase production caused by a group F phage was demonstrated in the same bacterial strain. The group A phage isolated from V4 induced the same lysogenic conversion to a higher production of P-V leucocidin in the nonlysogenic strain S57. This strain could be converted to gain of staphylokinase and a loss of beta toxin production by lysogenization with the F phage derived from strain V4.

**LITERATURE CITED**


