observations made with ^{3}H-labeled FIT suggest that a single molecular weight class of protein is specifically labeled in NG108-15 membranes (*11*).

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Toxic Shock Syndrome and Lysogeny in *Staphylococcus aureus*

Abstract. *Lysogeny, or the presence of temperate bacteriophage, was demonstrated, by means of two* Staphylococcus aureus *indicator strains, in 11 of 12 strains of* S. aureus *isolated from patients with toxic shock syndrome. Only 1 of 18 strains of* S. aureus *that were not associated with toxic shock syndrome showed the presence of bacteriophage. A laboratory strain of* S. aureus *was lysogenized by bacteriophage from two of the toxic shock–associated strains. These results add support to the theory that lysogeny by one or more bacteriophage in certain strains of* S. aureus *may be responsible for the pathogenesis of toxic shock syndrome.*

The toxic shock syndrome (TSS) is associated with *Staphylococcus aureus* and is characterized by fever, hypotension, desquamating rash, as well as other symptoms (*1, 2*). The syndrome has occurred in menstruating and nonmenstruating women, men, and children and has been associated with tampon use at menstruation, as well as staphylococcal empyema, septic abortions, fasciitis, osteomyelitis, and abscesses (*3, 4*). Although the syndrome may have occurred in the past (*5*), an increased incidence of the disease particularly in menstruating young women has only been noticed in the past 4 years.

According to the work of several investigators, the various symptoms and signs of TSS could be explained by the actions of a toxin. Two toxins associated with the extracellular products of *S. aureus* in TSS are enterotoxin F (*6*) and pyrogenic exotoxin C (*7*). Pyrogenic exotoxin C is believed to be a form of enterotoxin F (*8*). Pyrogenic exotoxin C is characterized by an isoelectric point of 7.2, a molecular weight of 22,000, pyrogenicity, enhancement of the effects of endotoxin, enhancement of a positive Dick test, suppression of immunoglobulin M synthesis after a second antigenic exposure, and ability to stimulate T cell proliferation (*8*).

Many of the signs and symptoms of TSS, such as fever, rash, and shock, are similar to those of scarlet fever in which the erythrogenic toxin of *Streptococcus pyogenes* plays a significant role. The production of the exotoxin of *S. pyogenes* (*9*) isolated in scarlet fever as well as the diphtheria toxin of *Corynebacterium diphtheriae* (*10*) have been clearly shown to be the result of lysogenic conversion. This term is used to describe a process by which genetic material of the bacteriophage is incorporated into the DNA of the bacterium, thereby conferring certain characteristics on the host strain. This is in contrast to virulent bacteriophage infection which results in complete lysis of the bacterium and release of viral progeny. The process of lysogenic conversion has been most extensively studied in the *Corynebacterium* in which a bacteriophage carrying the toxin gene is able convert a non-toxin–producing bacterium to a toxin producer [for a review, see (*11*)].

On the basis of these observations, we postulated that the toxin (or toxins) responsible for TSS may be under the same genetic control. In this report we present evidence that a significant number of TSS-associated *S. aureus* strains are lysogenized by one or more particular bacteriophage that are not found in non-TSS–associated strains, and that bacteriophage derived from the TSS-associated strains can be used to lysogenize another strain of *S. aureus*.

Strains of *S. aureus* associated with cases of TSS meeting the Centers for Disease Control (CDC) criteria for the disease were obtained from (i) CDC, Atlanta, Georgia, (ii) isolates from patients seen by us, (iii) New York Hospital–Cornell Medical Center, and (iv) samples sent by J. A. Giron and S. E. Read (Table 1). Non-TSS–associated strains, including vaginal isolates from asymptomatic women, were provided by some of the same sources. The *S. aureus* indicator strains 450, 450N (novobiocin-resistant), and 1830 were provided by S. Schaefler of the Public Health Research Institute of the City of New York.

Todd-Hewitt broth (*12*) was used to grow the organisms. Medium for agar plates (1 liter) consisted of 3 g of Casamino acids (Difco, Technical), 3 g of yeast extract (Difco), 5.9 g of NaCl, 20 ml of 2.5*M* Trizma base–HCl, *p*H 7.8, and 15 g of agar, to which 5 ml of 0.8*M* $CaCl_2$ was added just before the plates were poured. After the plates had solidified, excess moisture was removed from the agar surface as described by Zabriskie (*9*).

Screening for the presence of phage in each staphylococcal strain was achieved by growing an overnight culture (diluted 1:50) at 37°C to an optical density of 0.18 to 0.22 (measured at 650 nm in a Coleman 44 Spectrophotometer in a 16 by 125 mm tube). One milliliter of this suspension was used to flood the surface of agar plates. After the plates were tilted to remove excess fluid, they were allowed to stand at room temperature for 15 minutes. The plates were then inverted with the covers slightly off in a dry incubator at 37°C for 30 to 60 minutes. Plates containing these bacterial lawns

Table 1. Detection of lysogenic bacteriophage in TSS-associated *S. aureus* strains. The strains were obtained from Rockefeller University (RU); Centers for Disease Control (CDC); New York Hospital–Cornell Medical Center (NYH); J. A. Giron, Queens Hospital Center Affiliation, Long Island Jewish Medical Center, Jamaica, New York; and S. E. Read, Hospital for Sick Children, Toronto, Ontario.

Strain	Indicator		Site	Source
	450	1830		
Sw	+	+	Vagina	RU
587	+	+	Vagina	CDC
1270	−	+	Vagina	CDC
1439	−	+	Vagina	CDC
189	−	−	Vagina	CDC
Re	+	+	Breast abscess	NYH
Bu	−	+	Back abscess*	Giron
CS	+	+	Vagina	NYH
1169	+	−	Vagina	NYH
19409	−	+	Vagina	Read
27073	−	+	Vagina	Read
033	+	−	Vagina	CDC

*Caused by bee sting.

were irradiated at a distance of 1 m for 30, 40, 50, and 60 seconds each, using a 15-watt ultraviolet bulb (GE Germicidal). Indicator strains (20 μl) grown to the same optical density as described above were placed both undiluted and at a 1:6 dilution (20 μl) on the irradiated bacterial lawns. The plates were incubated at 37°C and examined after 18 hours. The presence of plaques in the indicator strains was used as evidence for release of bacteriophage from the irradiated strain.

Phage stocks from TSS-associated strains CS and Sw were obtained by a similar process with the use of multiple drops of the sensitive indicator on each irradiated plate. The phage were harvested by washing the surface of each plate with 1 to 2 ml of Todd-Hewitt broth. Excess bacteria were removed by centrifugation for 3 minutes in a Microfuge B and filtration through a 0.45-nm Millipore filter. Bacterial contamination was assayed by plating 0.1 ml of the phage stock on blood agar plates and incubating overnight at 37°C. Phage obtained from strains CS and Sw were termed CSϕ and Swϕ, respectively.

The novobiocin-resistant 450N indicator strain of *S. aureus* was prepared on agar plates as above. Twenty microliters of phage stocks CSϕ and Swϕ were placed on the surface of the bacterial lawn. After 18 hours at 37°C, the center of the clear zone was stabbed with a sterile needle and inoculated into Todd-Hewitt broth to select for a lysogenized strain. Serial dilutions of the overnight growth were made in Todd-Hewitt broth and streaked on the surface of novobiocin plates. Individual colonies were selected and tested for the presence of lysogenic bacteriophage by (i) resistance to superinfection by phage from the lysogenizing phage stock and (ii) their ability to release bacteriophage after irradiation, as described, using strain 450N as an indicator.

Eleven of twelve (92 percent) TSS-associated strains, upon irradiation, were able to yield plaques on at least one of the indicator strains, 450 and 1830. Seventeen of eighteen (94 percent) non-TSS–associated strains, including four vaginal isolates from young adults, did not release bacteriophage to which these indicators were sensitive. This difference was significant as tested by χ^2 analysis ($P < .001$) (Tables 1 and 2).

To determine whether the phage isolated from TSS-associated strains could lysogenize a sensitive *S. aureus* strain, we used strains CS and Sw, which were associated with menstrual tampon-related TSS in two women, to obtain phage stocks. These stocks were used to lysogenize strain 450N, a novobiocin-resistant mutant (chromosomal) of the 450 indicator. The resultant lysogenized strains were designated 450N(CSϕ) and 450N(Swϕ).

Lysogeny of both 450N(CSϕ) and 450N(Swϕ) strains was demonstrated by the release of bacteriophage and subsequent plaque formation on the 450N indicator (Table 3). Each lysogenized strain was also resistant to both phage stocks, CSϕ and Swϕ, as were the original CS and Sw parent strains. Since CS and Sw were both sensitive to novobiocin, contamination of strains 450N(CSϕ) and 450N(Swϕ) with the parental strains was ruled out by their growth on novobiocin-containing agar plates.

Table 2. Detection of bacteriophage in non-TSS–associated *S. aureus* strains.

Strain	Indicator		Site	Source
	450	1830		
4214	−	−	Vagina	CDC
4161	−	−	Vagina	CDC
Be	−	−	A-line tip	NYH
Fa	−	−	Stitch abcess	NYH
Ha	−	−	Phlebitis	NYH
Li	−	−	Transplant biopsy	NYH
We	−	−	Back	NYH
Se	−	−	Nose (sinusitis)	NYH
0318	−	−	Vagina	CDC
4159	−	−	Vagina	CDC
De	−	−	Jugular	NYH
Ir	−	−	Wound	NYH
Je	−	−	Face	NYH
Ki	−	−	Finger	NYH
Da	−	−	Unknown*	NYH
Ja	−	−	Unknown*	NYH
Mi	+	+	Sputum	NYH
Br	−	−	Unknown*	NYH

*Nonvaginal source.

Table 3. Lysogenicity of 450N indicator.

Strain	Detection of phage by irradiation with 450N indicator	Production of plaque by phage lysates	
		CSϕ	Swϕ
CS	+	−	−
Sw	+	−	−
450N	−	+	+
450N(CSϕ)	+	−	−
450N(Swϕ)	+	−	−

The observation that lysogeny occurs in most TSS strains of *S. aureus* brings to mind the parallel association of lysogeny with the production of diphtheria toxin in *C. diphtheriae* and erythrogenic toxin in *S. pyogenes*. The similarities between the clinical signs of TSS and scarlet fever further advance the concept of a causal relation between lysogeny and toxin production in TSS strains.

The sensitivity of a bacterial strain to phage infection depends on several factors of which the presence of surface receptors and absence of lysogenization by a related bacteriophage are of major importance. With respect to staphylococci, lysogeny among strains of *S. aureus* is common (*13, 14*), and the extent to which lysogenization is detected within these strains depends on the number of indicators used. *Staphylococcus aureus* strains have also been found to be lysogenized by more than one type of bacteriophage (*15–17*). Such strains are said to be poly-lysogenized.

When two indicator strains were used to screen *S. aureus* strains isolated from both TSS-associated and non-TSS–associated cases, 92 percent (11 out of 12) were found to contain temperate bacteriophage that could infect one or both indicators. This was in sharp contrast to the results from the non-TSS–associated strains of *S. aureus* in which 94 percent (17 out of 18) were negative for phage capable of infecting these same indicators. Given the high degree of lysogeny among *S. aureus* strains, it was somewhat unexpected that TSS-associated and non-TSS–associated strains could be differentiated on the basis of these two indicator strains. This suggests related characteristics of the lysogenic phage in these strains and the presence of specific receptors for this particular group of bacteriophage. Alternatively, as TSS-associated strains have frequently been of group I phage type (*18*), our indicators may reflect a higher degree of sensitivity to lysogenic phage from this group. In any event, these experiments show that

a significant number of *S. aureus* strains from patients with TSS possess one or more common bacteriophage and that these phage can be transferred to another strain to yield a newly lysogenized bacterium. Another concept, namely that plasmids per se may be involved in TSS-associated strains, has been diminished by Kreiswirth *et al.* (*19*), who demonstrated a lack of association of plasmids with previously described toxin (or toxins) associated with TSS.

The question of whether or not lysogenic bacteriophage in TSS-associated strains of *S. aureus* are responsible for the production of pyrogenic exotoxin C/enterotoxin F, or perhaps a toxin or toxins not yet described, is unanswered. Blair and Carr (*14*) have already demonstrated that bacteriophage from an α-hemolysin–producing *S. aureus* strain could lysogenize another strain of *S. aureus* and that this was accompanied by a new capacity to produce α-hemolysin. Further experiments should show whether or not lysogenization of certain *S. aureus* strains with particular bacteriophage capable of inducing toxin formation plays the crucial role in initiating the pathological events that lead to toxic shock syndrome.

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Human Leukocyte Functions and the U.S. Safety Standard for Exposure to Radio-Frequency Radiation

Abstract. *Human mononuclear leukocytes were exposed to microwaves at energies relevant to current public safety recommendations. No detectable effects on viability or function of the leukocytes resulted from exposure to microwaves at specific absorption rates up to 4 milliwatts per milliliter. The results were highly reproducible and provided no evidence that current safety standard recommendations are inappropriate insofar as leukocyte function is concerned.*

All individuals are exposed to radiofrequency or microwave energies to variable degrees. Studies by several investigators have raised the possibility that the immunocompetent cells of humans are particularly susceptible to microwaves (*1*). These studies were admitted by at least some of the authors to be poorly reproducible and nonquantitative. Nonetheless, they are frequently cited, and they have provided the limited data available, on exposure of human leukocytes, for use by the individuals and agencies that develop environmental health standards (*2*). Many animal systems have been studied, but the species, microwave power intensities, environmental conditions, and other factors have been so varied that extrapolation to humans would be exceedingly difficult, even if appropriate (*3, 4*).

The studies reported here provide data on exposure of human leukocytes to microwave energies relevant to current public safety recommendations. Exposure to microwaves at specific absorption rates up to 4 mW/ml resulted in no detectable effects on viability or on unstimulated or stimulated DNA, RNA, total protein, or interferon synthesis by human mononuclear leukocytes. In contrast to the studies cited above, the results were highly reproducible and provided no evidence that current safety standard recommendations are inappropriate.

Human mononuclear leukocytes were exposed in a waveguide system (*5*) to 2450-MHz (continuous-wave) microwaves for 2 hours at specific absorption rates (SAR's) of 0.5 to 4 mW/ml and were subsequently incubated in a humidified CO_2 incubator at 37°C. The safety standard limit proposed by Committee C-95.4 of the American National Standards Institute is 0.4 mW/g, which is equivalent in these cultures of 0.4 mW/ml. This standard incorporates approximately a tenfold safety factor relative to the basal metabolic rate for humans (3.5 mW/g). In addition to leukocyte cultures enclosed within waveguides for exposure or sham-exposure, we included control cultures located in the same incubator but external to the waveguides. No attempt was made to counteract microwave-induced heating of the leukocyte cultures since we wished to observe any potential microwave-induced effects, thermal or otherwise. Exposure of the leukocytes at an SAR of 4 mW/ml produced no significant changes in cell viability for up to 1 week after exposure (Table 1); results were similar with exposures at lower SAR's.

Unstimulated and mitogen-stimulated DNA, RNA, and total protein synthesis was examined after exposure of the mononuclear leukocytes to microwaves at SAR's of 4 mW/ml or less. There were no significant differences between microwave-exposed (4 mW/ml), sham-exposed, and control leukocytes in unstimulated nucleotide or protein synthesis, or in responses of the leukocytes to an optimal concentration of mitogen (Fig. 1). Similar results were obtained with suboptimal concentrations of mitogen and lower SAR's (0.5 and 1.0 mW/ml) for the microwave-exposed cultures (data not shown). Microscopic inspection of Wright-Giemsa–stained cytospin preparations (*6*) did not reveal any discrepancies between morphologic lymphocyte blastogenesis (used in some of the studies cited earlier) and determinations based on incorporation of the radiolabeled precursors.

Table 1. Total viable mononuclear leukocytes after exposure to microwave energy at an SAR of 4 mW/ml. Each value is the mean total number of viable cells (total cells × percent viable) × 10^{-4}, ± standard error. Insufficient numbers of observations (< 5) were available 3 days after exposure. Viability was assessed by the ability of the cells to exclude trypan blue dye and ethidium bromide.

Days after exposure	Mean total number of viable cells		
	Microwave	Sham	Control
1	58 ± 7	65 ± 11	54 ± 6
2	60 ± 9	63 ± 15	56 ± 6
4	41 ± 4	39 ± 2	41 ± 8
5	47 ± 16	72 ± 36	46 ± 13
6	39 ± 10	41 ± 9	36 ± 7
7	40 ± 10	37 ± 8	38 ± 11