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Activation of the human immunodeficiency virus long terminal repeat in THP-1 cells by a staphylococcal extracellular product

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ABSTRACT **Staphylococcal strains can release a factor that strongly activates the human immunodeficiency virus type 1 (HIV-1) long terminal repeat (LTR) in THP-1 cells transfected with the HIV-1 LTR-driven luciferase reporter gene (THP-1 LTR_{luc}). The factor is present in the overnight culture fluid and is readily released from the organisms into aqueous medium by vigorous mixing. Staphylococcal extracellular material is a complex mixture of polysaccharide and protein containing peptidoglycan and teichoic acid, released in part by cell wall turnover. The importance of the carbohydrate component is emphasized by concanavalin A (Con A) inhibition of staphylococcal product-induced LTR activation but not of activation by phorbol 12-myristate 13-acetate or tumor necrosis factor. The effect of Con A was decreased or abolished by sugars in the order methyl α-D-mannopyranoside > methyl α-D-glucopyranoside > mannose > glucose = fructose > *N*-acetylglucosamine. Wheat germ agglutinin was less inhibitory than Con A; in this instance *N*-acetylglucosamine decreased inhibition, whereas methyl α-D-mannopyranoside or methyl α-D-glucopyranoside did not. The induction of luciferase activity in THP-1 LTR_{luc} by the staphylococcal extracellular product also was inhibited by fetal bovine and normal human serum. A comparison of 31 staphylococcal isolates (9 *Staphylococcus aureus*, 11 *Staphylococcus epidermidis*, 2 *Staphylococcus haemolyticus*, 4 *Staphylococcus hominis*, 2 *Staphylococcus capitis*, 2 *Staphylococcus warneri*, 1 *Staphylococcus saprophyticus*) revealed wide variation in LTR activating activity that did not correlate closely with slime production. Our findings, using induction of luciferase in THP-1 LTR_{luc} as a model for upregulation of HIV infection, raise the possibility that staphylococci, as well as certain other microorganisms, release carbohydrate-containing exopolymers, which can activate the HIV-1 LTR, thus influencing progression of HIV infection.**

The long terminal repeat (LTR) of human immunodeficiency virus type 1 (HIV-1) contains sequences that respond to a number of cis or trans activating factors to initiate gene expression and viral replication (1, 2). We have initiated a study of this process by the integration of a construct, consisting of the luciferase reporter gene under the control of the HIV-1 LTR, into the monomyelocytic cell line THP-1. Activation of the LTR leads to luciferase production, which can be readily measured. We report here that a staphylococcal extracellular product strongly activates the HIV-1 LTR in the THP-1 cell line.

MATERIALS AND METHODS

Construction of THP-1 LTR_{luc}. A plasmid ($pHIV_{lai}$) containing the HIV_{lai} genome modified by deletion of a portion of the *gag* and *pol* genes (kindly provided by Michael Emerman, Fred Hutchinson Cancer Research Center, Seattle) was cut with *Acc*651 and *Hin*dIII releasing a 598-bp fragment containing most of the 3′ LTR. The fragment, which contained 66 bases 5′ to the beginning of the LTR and was missing 19 bases at the 3′ end, was ligated into the *Acc*651 and *Hin*dIII sites upstream to the luciferase gene in the pGL2-Basic vector (Promega). The plasmid was linearized using *Bam*HI and *Sca* I sites and cotransfected with the neomycin phosphotransferase II gene (RSV-Neo; kindly provided by Michael Emerman) into THP-1 cells by electroporation (ECM-600 electroporator, BTX, San Diego; settings: voltage, 100 V; capacitance, 2950 μF; resistance, 6 Ω). Stable transfectants were selected in G418 (750 μg of Geneticin per ml; GIBCO) and cloned by limiting dilution. Transfection of THP-1 LTR_{luc} with the *tat* gene (pCMV-tat; kindly provided by Michael Emerman) increased the luciferase activity from a basal rate of 800–1500 to 100,000–120,000 relative light units (RLU). The cells were maintained in RPMI 1640 medium containing 25 mM Hepes buffer, 2 mM L-glutamine, 50 units of penicillin per ml, 50 μg of streptomycin sulfate per ml (BioWhittaker), 500 μg of G418 sulfate per ml, and 10% heat-inactivated fetal bovine serum (FBS; GIBCO) (RPMI/FBS). They were split 1:1 weekly and used 2–5 days later. On the day of the experiment, unless otherwise indicated, the cells were washed once and suspended in RPMI 1640 medium containing Hepes buffer and L-glutamine but without antibiotics, G418, or FBS (RPMI).

Staphylococcal Strains. Twenty-seven staphylococcal clinical isolates were kindly provided by the University of Washington Medical Center Clinical Microbiology Laboratory. The isolates, speciated by the Staph-Ident staphylococcal system (Analytlab Products, Plainview, NY), consisted of 9 *Staphylococcus aureus* (*S. aureus* UW1-9), 9 *Staphylococcus epidermidis* (*S. epi* UW1-9), 2 *Staphylococcus haemolyticus* (*S. haem* UW1 and -2), 2 *Staphylococcus hominis* (*S. hom* UW1 and -2), 2 *Staphylococcus capitis* (*S. cap* UW1 and -2), 2 *Staphylococcus warneri* (*S. warn* UW1 and -2), and 1 *Staphylococcus saprophyticus* (*S. sapro* UW1). Four ATCC strains (American Type Culture Collection), *S. epi* 35983 and 35984 and *S. hom* 35981 and 35982, were also used.

The staphylococcal strains were maintained on T Soy agar plates and were transferred to T Soy broth for overnight growth prior to the experiment. The culture medium was obtained by centrifugation of the overnight culture twice at 1500 × *g*. Unless otherwise indicated, the organisms were washed twice with RPMI and suspended in RPMI to A_{540} 1.0 [8×10^6 colony-forming units (cfu)/ml] when intact staphylococci were used or to A_{540} 5.0 (1.6×10^9 cfu/ml) for preparation of extracts. The extracts were prepared by vigorous Vortex mixing of the bacterial suspension in RPMI at room temperature for 1 min and collection of the supernatant by centrifugation twice at 1500 × *g*.

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Abbreviations: cfu, colony-forming units; FBS, fetal bovine serum; HIV-1, human immunodeficiency virus type 1; LTR, long terminal repeat; PMA, phorbol 12-myristate 13-acetate; RLU, relative light units; TNF-α, type α tumor necrosis factor; WGA, wheat germ agglutinin; NS, not significant.

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Measurement of Luciferase Activity. Luciferase activity was determined with a luciferase assay kit (Promega) and the photons, counted over a 30-sec period, were designated as RLU. A luminometer (Monolight 1500; Analytical Luminescence Laboratory, San Diego) was used.

Measurement of Slime Production. Slime production by the staphylococcal strains was measured essentially as described (3, 4). Into the wells of 96-well polystyrene plates (Costar 3595) was placed 0.2 ml of an overnight culture of the staphylococcal strain that had been washed and resuspended in fresh T Soy broth at 10×10^6 cfu/ml. The plates were incubated overnight at 37°C, the broth was gently aspirated, and the wells were washed twice with phosphate-buffered saline. The material adherent to the surface of the wells was fixed by 5-min exposure to Bouin's fixative, stained with 0.5% safranin for 1 min, and washed twice with water; the absorbance was determined at 490 nm against a blank consisting of T Soy broth without organisms similarly processed, using an automated microplate reader [Bio-Tek (Burlington, VT) model EL309].

Statistical Analysis. The Mann–Whitney one-tailed rank sum *U* test was used to analyze differences for significance [$P > 0.05$, not significant (NS)].

RESULTS

Table 1 demonstrates the activation of the HIV-1 LTR in THP-1 cells by staphylococcal products. The organism used was a strain of *S. epidermidis* designated *S. epi* UW3. In contrast to uninfected T Soy broth, the addition of culture medium (final concentration, 5%) increased luciferase activity 27-fold above background. Intact *S. epi* UW3 also greatly increased luciferase activity (50-fold). The number of viable organisms present in the reaction mixture initially was 8.2×10^6 per ml and this number increased to 1.1×10^8 per ml during the 6-h incubation period. The *S. epi* UW3 extract retained the ability to activate luciferase production, with 5% extract increasing activity 81-fold. The organisms were washed twice with RPMI with gentle mixing by inversion in order to remove culture medium components prior to preparation of the extract. Each of the wash fluids also was active in inducing luciferase activity (data not shown). A different THP-1 LTR_{luc} clone produced by DEAE-dextran transfection responded similarly to the *S. epi* extract (median RLU background, 1781; *S. epi* UW3 extract, 180,337; $n = 3$; $P = 0.05$). Activation of THP-1 LTR_{luc} was induced by endotoxin (*Escherichia coli* 055:B5; Sigma) only at concentrations >1 μg/ml, levels that were orders of magnitude greater than those added in any of the reagents.

Fig. 1 demonstrates the time course of the induction of luciferase activity by the *S. epi* UW3 extract. In a reaction mixture in which the THP-1 LTR_{luc} was suspended in RPMI with antibiotics and G418 but without FBS, activity increased

Table 1. Activation of the HIV LTR in THP-1 cells by staphylococcal products

Addition	Luciferase activity, RLU	*P*
None	1,220 (26)	
Uninfected T Soy broth	1,278 (3)	NS
Culture medium	42,022 (6)	<0.001
S. epi	86,558 (21)	<0.001
S. epi extract	113,728 (12)	<0.001

Reaction mixture contained 2×10^6 THP-1 LTR_{luc} and, where indicated, 100 μl of T Soy broth, 100 μl of *S. epi* UW3 culture medium, 8.2×10^6 *S. epi*, and 100 μl of *S. epi* extract in RPMI at a final volume of 2.0 ml. Results are the median of (*n*) experiments with the *P* value indicating the significance of the difference from background (None).

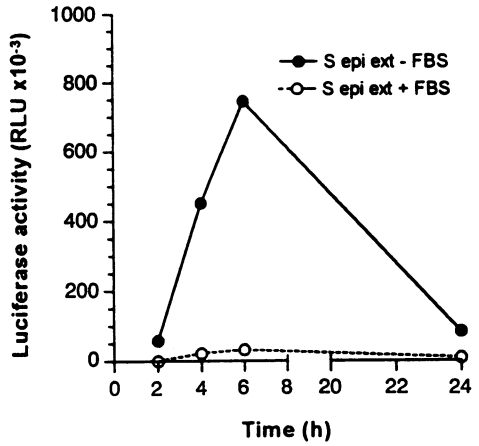

FIG. 1. Time course of the induction of luciferase activity by the *S. epi* UW3 extract. To tissue culture flasks (25102-25, Corning) were added 12×10^6 THP-1 HIV_{luc} and, where indicated, 5% *S. epi* UW3 extract and either RPMI or RPMI/10% FCS to a final volume of 12 ml. Incubation was at 37°C in a CO_2 incubator. At the times indicated, 2 ml of the reaction mixture (2×10^6 THP-1 cells) was removed for determination of luciferase activity. Results are median of three to five experiments.

to a maximum at 6 h and then decreased as the incubation was extended to 24 h. A similar time course was observed when the cells were suspended in RPMI that also contained 10% FBS; however, the extent of the stimulation was markedly depressed. All subsequent experiments were performed with RPMI without FBS.

Con A is a plant lectin with a high affinity for α-D-mannosyl and α-D-glucosyl residues. It is converted from a tetrameric to a dimeric form by succinylation without alteration in carbohydrate binding specificity but with alterations in biological activity due to the reduced valence (5). Activation of the LTR by the *S. epi* extract was inhibited by Con A, with a significant inhibition (89%) observed at 30 μg/ml, which increased to 97.7% at 100 μg/ml and 97.8% at 300 μg/ml (Fig. 2). Con A (100 μg/ml) alone—i.e., in the absence of the *S. epi* extract—significantly increased luciferase activity from a median background RLU of 1530 to a value of 4990 ($n = 8$; $P < 0.005$). Succinyl Con A was considerably less inhibitory,

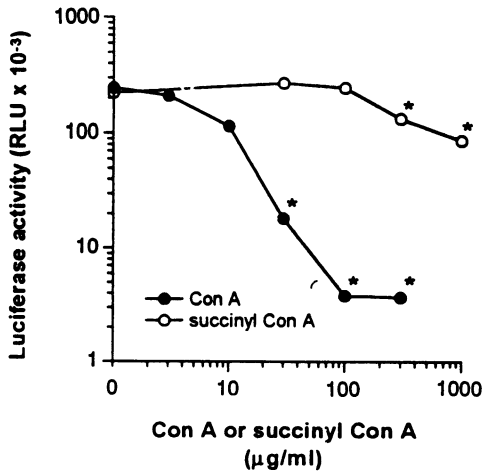

FIG. 2. Inhibition of *S. epi* extract-induced activation of the LTR by Con A. Reaction mixture contained 2×10^6 THP-1 LTR_{luc}, 100 μl of *S. epi* UW3 extract, and Con A (●) or succinyl Con A (○) at the concentrations indicated in RPMI at a final volume of 2.0 ml. Results are the median minus background (RLU, 1498) of three to eight experiments. Asterisk indicates a significant difference from no Con A or succinyl Con A ($P \leq 0.05$).

with a significant inhibition observed at 300 μg/ml (40.1%), which increased to 61.1% at 1000 μg/ml. The inhibition by Con A of the *S. epi* extract-induced activation of the LTR was partially or totally prevented by sugars in the rank order methyl α-D-mannopyranoside > α-D-glucopyranoside > mannose > glucose = fructose > *N*-acetylglucosamine, with methyl α-D-galactopyranoside, galactose, and fucose being ineffective under the conditions used in Table 2. The lectin from *Triticum vulgaris* [wheat germ agglutinin (WGA); Sigma] also inhibited the activation of the LTR by the staphylococcal product; in this instance, the inhibition was unaffected by methyl α-D-mannopyranoside and methyl α-D-glucopyranoside (0.003–0.03 M) but was partially prevented by *N*-acetylglucosamine at 0.03 M (Table 2). Con A and WGA had no significant effect on the activation of the LTR by phorbol 12-myristate 13-acetate (PMA; Sigma) or tumor necrosis factor α (TNF-α; Genentech) under the conditions used in Table 3.

Table 2. Prevention of Con A inhibition of *S. epi*-induced activation of the LTR by certain sugars

Addition(s)	Luciferase activity, RLU	(*n*)	*P*
None	1,856	(14)	
S. epi extract	178,172	(14)	
E. epi extract + Con A	7,618	(14)	
S. epi extract + WGA	26,214	(5)	
S. epi extract + Con A + 0.003 M sugars			
Methyl α-D-mannopyranoside	66,180	(3)	<0.002
Methyl α-D-glucopyranoside	24,097	(3)	<0.002
Methyl α-D-galactopyranoside	6,385	(3)	NS
Mannose	21,004	(3)	<0.005
Glucose	9,299	(3)	NS
Galactose	4,595	(3)	NS
Fructose	6,901	(3)	NS
Fucose	6,861	(3)	NS
N-Acetylglucosamine	8,359	(3)	NS
S. epi extract + WGA + 0.003 M sugars			
Methyl α-D-mannopyranoside	25,902	(4)	NS
Methyl α-D-glucopyranoside	26,261	(4)	NS
N-Acetylglucosamine	28,593	(5)	NS
S. epi extract + Con A + 0.01 M sugars			
Methyl α-D-mannopyranoside	191,214	(6)	<0.001
Methyl α-D-glucopyranoside	80,712	(3)	<0.002
Methyl α-D-galactopyranoside	6,074	(3)	NS
Mannose	43,543	(3)	<0.002
Glucose	14,991	(3)	<0.01
Galactose	4,949	(3)	<0.02
Fructose	14,259	(3)	<0.01
Fucose	7,488	(3)	NS
N-Acetylglucosamine	10,286	(3)	NS
S. epi extract + WGA + 0.01 M sugars			
Methyl α-D-mannopyranoside	25,916	(4)	NS
Methyl-α-D-glucopyranoside	22,271	(4)	NS
N-Acetylglucosamine	36,156	(5)	NS
S. epi extract + Con A + 0.03 M sugars			
Methyl α-D-mannopyranoside	240,766	(3)	<0.002
Methyl α-D-glucopyranoside	121,285	(3)	<0.002
Methyl α-D-galactopyranoside	8,229	(3)	NS
Mannose	145,739	(3)	<0.002
Glucose	21,142	(3)	<0.002
Galactose	3,707	(3)	NS
Fructose	46,263	(3)	<0.002
Fucose	6,154	(3)	NS
N-Acetylglucosamine	19,970	(3)	<0.002
S. epi extract + WGA +0.03 M sugars			
Methyl α-D-mannopyranoside	26,805	(4)	NS
Methyl α-D-glucopyranoside	20,648	(4)	NS
N-Acetylglucosamine	100,712	(5)	<0.01

Reaction mixture contained 2 × 10^6 THP-1 LTR_{luc} and, where indicated, 100 μl of *S. epi* UW3 extract, 100 μg of Con A per ml, 100 μg of WGA per ml, and the sugars at 0.003, 0.01, or 0.03 M as indicated in a final volume of 2.0 ml. Results are median RLU of (*n*) experiments with the *P* value indicating the significance of the difference from *S. epi* extract + Con A or WGA.

Table 3. Effect of Con A and WGA on activation of the LTR by PMA or TNF-α

Addition(s)	Luciferase activity, RLU	*P**	*P*†
None	2,206		
PMA	18,800	<0.02	
PMA + Con A	18,878	<0.02	NS
PMA + WGA	32,379	<0.02	NS
TNF-α	20,125	<0.02	
TNF-α + Con A	35,886	<0.02	NS
TNF-α + WGA	25,690	<0.02	NS

Reaction mixture was as described in Table 1 except that PMA (100 ng/ml), TNF-α (100 units/ml), Con A (100 μg/ml), and WGA (100 μg/ml) were added where indicated. Results are the median of four experiments.
*Significance of difference from background (None).
†Significance of difference from either PMA or TNF-α alone.

Activation of the HIV-1 LTR by the staphylococcal product was strongly inhibited by normal human serum. Fifty percent inhibition was produced by 0.2% pooled serum and the inhibition increased to 98.5% on the addition of 10% serum (Fig. 3).

Table 4 compares the LTR activating activity and slime production of 31 strains of staphylococci (9 *S. aureus*, 11 *S. epi*, 2 *S. haem*, 4 *S. hom*, 2 *S. cap*, 2 *S. warn*, and 1 *S. sapro*). Extracts from all the strains tested were active in the induction of luciferase activity ($P \leq 0.05$ compared to background); however, considerable variation between strains was observed. The range of activity was from a low of 2741 above background (*S. sapro* UW1) to a high of 325,242 above background (*S. warn* UW1). Three extracts gave values below 10,000 (1 *S. sapro*, 1 *S. hom*, and 1 *S. epi*), 17 extracts gave values of 10,000–100,000 (7 *S. aureus*, 6 *S. epi*, 1 *S. haem*, and 3 *S. hom*), and 11 extracts gave values >100,000 (2 *S. aureus*, 4 *S. epi*, 1 *S. haem*, 2 *S. cap*, and 2 *S. warn*).

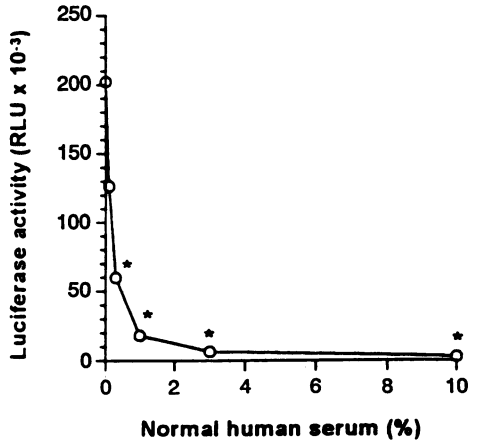

FIG. 3. Inhibition of *S. epi* extract-induced activation of the LTR by normal human serum. Reaction mixture contained 2 × 10^6 THP-1 LTR_{luc}, 100 μl of *S. epi* UW3 extract, and normal human serum at the concentrations indicated in RPMI at a final volume of 2.0 ml. Results are the median minus background (RLU, 1050) of three experiments. Asterisk indicates a significant difference from the absence of serum—i.e., *S. epi* extract alone ($P \leq 0.05$).

Table 4. Comparison of slime productivity and induction of luciferase activity by extracts of various staphylococcal strains

Staphylococcal strain	Luciferase activity, RLU	Slime production, A_{490}
S. aureus		
UW1	69,703	−0.039
UW2	14,741	0.129
UW3	57,922	0.148
UW4	64,073	0.351
UW5	121,240	0.096
UW6	28,882	0.112
UW7	22,887	0.105
UW8	108,251	0.040
UW9	30,872	0.170
S. epidermidis		
UW1	14,893	0.186
UW2	34,803	>2.5
UW3	223,087	0.391
UW4	240,005	0.451
UW5	201,826	1.038
UW6	4,857	0.075
UW7	64,037	0.217
UW8	97,819	>2.5
UW9	217,970	0.801
ATCC 35984	46,328	>2.5
ATCC 35983	57,148	0.211
S. haemolyticus		
UW1	107,589	−0.032
UW2	11,976	−0.105
S. hominis		
UW1	23,765	0.006
UW2	20,692	2.406
ATCC 35981	17,220	0.048
ATCC 35982	3,431	0.031
S. capitis		
UW1	208,258	0.784
UW2	135,249	0.151
S. warneri		
UW1	325,242	1.210
UW2	115,249	0.100
S. saprophyticus		
UW1	2,741	0.515

Reaction mixture was as described in Table 1 (*S. epi* extract) for measurement of luciferase induction, and slime production was measured as described, with the staphylococcal strains being varied as indicated. Results for luciferase activity are the median above background (RLU, 1259) of three experiments and results for slime production are the median of three to seven experiments with an upper limit of the assay of 2.5.

The average slime production of the 9 *S. aureus* strains was 0.119 and that of the 11 *S. epi* strains was >1.005 (3 *S. epi* strains with values >2.5 were designated as 2.5 for averaging purposes), whereas the average luciferase activity was 57,619 for the *S. aureus* and 109,343 for the *S. epi* strains. The average luciferase activity for the four strains with slime production >2.4 (*S. epi* UW2, *S. epi* UW8, *S. epi* ATCC 35984, and *S. hom* UW2) was 49,911, whereas the four strains with no detectable slime production (*S. aureus* UW1, *S. haem* UW1 and UW2, and *S. hom* UW1) averaged 53,258 in the luciferase assay. Thus, our studies with 31 staphylococcal strains did not reveal a close relationship between slime production as measured by biofilm formation and the ability of the extract to activate the HIV LTR as measured by the induction of luciferase activity.

DISCUSSION

A substance (or substances) is released by some staphylococcal strains that activate the LTR of HIV-1 as measured by the induction of luciferase synthesis in THP-1 cells transfected with HIV-1 LTR_{luc}. The finding that two different clones from separate transfections both show induction of luciferase by the staphylococcal extract argues strongly against this activation being due to cis-acting elements at the site of integration of LTR_{luc}. This staphylococcal extracellular product is found in the culture fluid and is readily released from organisms by shaking in aqueous medium, indicating that although it can remain in part associated with the bacterial surface, it is not covalently bound. The active material contains an essential carbohydrate component since its induction of luciferase activity in THP-1 cells is strongly inhibited by the lectin Con A, an effect that is partially or totally prevented by sugars in the order methyl α-D-mannopyranoside > methyl α-D-glucopyranoside > mannose > glucose = fructose > *N*-acetylglucosamine. The inhibitory effect of Con A is markedly reduced but not lost by its conversion to the dimeric form by succinylation. WGA also was inhibitory, although to a lesser degree than Con A; this inhibition was unaffected by methyl α-D-mannopyranoside and methyl α-D-glucopyranoside but was reduced by *N*-acetylglucosamine. These findings are compatible with the sugar specificities of the lectins. The lack of an effect of Con A and WGA on the activation of the LTR by PMA or TNF-α indicated that the lectins were not generally inhibitory under the conditions used.

The cell wall of staphylococci contains a peptidoglycan polymer composed of repeating linear β(1–4)-linked *N*-acetylmuramic acid and *N*-acetylglucosamine subunits containing tetrapeptide side chains (L-alanine, D-isoglutamine, L-lysine, D-alanine) linked to the muramic acid and crosslinked usually by a pentaglycine bridge connecting the L-lysine of one chain to the D-alanine of another. Teichoic acids are additional major components of the cell wall of staphylococci. They can be covalently linked to the muramic acid of the peptidoglycan either directly or via a small linkage unit (cell wall teichoic acid) or they can contain lipid (lipoteichoic acid) and associate with the membrane by hydrophobic interactions. The teichoic acid consists of repeating units of either ribitol or glycerol phosphate, which are glucosylated and contain ester-linked D-alanine residues. The staphylococcal components found external to the cell wall can include a variety of toxins, exoenzymes, and polysaccharide-containing exopolymers. The latter have been variously termed capsule, slime, or glycocalyx. The term capsule implies the maintenance of firm attachment to the organism. Slime, while often used as a general term for the extracellular products of staphylococci (6), is used here in a more restricted sense to describe the viscous extracellular material loosely associated with the organism that coats surfaces as a biofilm containing trapped organisms, a property we have used for its measurement. Glycocalyx refers to all polysaccharide-containing material external to the cell wall.

Essentially all staphylococci release complex polysaccharide-containing exopolymers due, in part, to autolytic enzyme-induced cell wall turnover, which can be as high as 25% per generation (7–10). In one study (8), analysis of the released material yielded products consistent with cleavage by *N*-acetylmuramic acid L-alanine amidase—i.e., muramic acid, glucosamine, alanine (including the N terminus), glutamic acid, lysine, glycine, and trace amounts of other amino acids, which may be contaminants. In some instances, this material is particularly viscous forming a biofilm (slime). Early analyses of the extracellular slime of coagulase-negative staphylococci revealed a polysaccharide rich in galactose; however, it was subsequently proposed that the source of the galactose-rich polysaccharide was the agar on which the organisms were grown (6, 11, 12). Analysis of slime isolated from *S. epi* strains grown in a chemically defined medium revealed a mixture of ≈80% carbohydrate and 20%

protein (13). The carbohydrate consisted of a single polymer containing glycerol phosphate, ester-linked D-alanine, glucose, and *N*-acetylglucosamine, suggesting that it was a glycerol teichoic acid (12, 13). No long-chain fatty acids were detected in the slime preparation, suggesting that the material was not a lipoteichoic acid (6). The presence of mannose in staphylococcal slime was proposed by Peters *et al.* (14), who separated *S. epi* extracellular material into four fractions on a DEAE-Sepharose column using a sodium chloride gradient. Mannose was the main sugar in fraction 1 and was also present in fraction 2, whereas it was absent from fractions 3 and 4. Mannose was not detected by Hussain *et al.* (12) in their preparations. The proteins of slime isolated from *S. epi* grown in a chemically defined medium were readily separated from the carbohydrate, suggesting that the material was not a glycoprotein (12) as earlier proposed (14). A requirement for both carbohydrate and protein for biofilm formation is suggested by its inhibition by either tunicamycin (an inhibitor of carbohydrate synthesis) or chloramphenicol (an inhibitor of protein synthesis) (15). The teichoic acid does not appear to totally account for the adhesive properties of slime since adherent and nonadherent strains of coagulase-negative staphylococci can form comparable amounts of teichoic acid (6). The staphylococcal extracellular product that activates the LTR is not synonymous with slime as defined here. Thus, the extracellular products released by some staphylococcal strains, while strongly inducing luciferase synthesis, do not form a heavy biofilm, and high slime producers need not be high inducers of luciferase activity.

The reaction of Con A as well as certain other lectins with staphylococcal surface or extracellular components has been well documented (12, 14, 16–18). Con A reacts selectively with α-D-mannopyranose or α-D-glucopyranose residues in which the 3-, 4-, and 6-hydroxyl groups are unsubstituted. The hydroxyl group at position 2, however, can be replaced by an acetamido group (e.g., *N*-acetylglucosamine) (19). WGA predominantly recognizes *N*-acetylglucosamine. Staphylococcal cell wall or extracellular components such as peptidoglycan, teichoic acid, and lipoteichoic acid or their autolytic products contain sugar residues that can react with these lectins. Archibald and Coapes (20) proposed that Con A reacts predominantly with α-D-glucopyranosyl residues in teichoic acids, whereas Reeder and Ekstedt (21) proposed the reaction of Con A with α-glycosylated teichoic acid in *S. epi* strains and to α-linked *N*-acetylglucosamine in strains of *S. aureus*. The detection of mannose residues in their *S. epi* slime preparations and the strong inhibitory effect of mannose on the interaction with Con A led Peters *et al.* (14) to propose the interaction of Con A with mannose residues. Hussain *et al.* (12), however, proposed that Con A reacts with glucose residues, since their *S. epi* slime preparation did not contain mannose yet reacted with Con A.

The induction of luciferase in THP-1 LTR_{luc} may serve as a model for upregulation of HIV infection in macrophages. Staphylococci, thus, may influence the progression of HIV infection through their release of components that affect viral transcription through activation of the LTR. Patients infected with HIV have an increased incidence of *S. aureus* infection (22–24), which appears to be related to an increased skin and mucous membrane carriage rate (25–27). Normal human serum strongly inhibited the activation of the LTR by staphylococcal products, as did FBS. This would argue against a role for staphylococcal products in locations where serum or serum components are present in large amounts. However, at skin and mucous membrane surfaces, where serum components are excluded or in low amounts and where staphylococci can reside, the influence of staphylococcal extracellular products on HIV replication needs to be considered. Although the studies reported here have been limited to the extracellular products of staphylococcal strains, other organisms produce comparable polysaccharides, which may similarly affect the HIV-1 LTR.

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