

Role of Keratinocyte Injury in Adherence of *Streptococcus pyogenes*

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Keratinocytes injured acutely by UVB light or lipopolysaccharide were used to test the hypothesis that keratinocyte injury promotes bacterial adherence and the development of group A streptococcal skin infections. Injury did not affect adherence to undifferentiated and differentiated keratinocytes, but keratinocyte differentiation promoted adherence four- to fivefold.

A widely held but poorly understood tenant of pyogenic skin infections is that cutaneous injury is necessary for colonization and infection to develop. Barrier function of the skin resides in the corneal layer (16, 30, 33), which presumably protects against microbial invasion mechanically and through its acidic pH, its dry environment, and the release of lipid breakdown products which are bactericidal (8, 10, 26) and may inhibit bacterial adherence (9). When *Streptococcus pyogenes* (group A streptococcus) is applied to intact skin of human volunteers or laboratory animals, infection fails to develop (our unpublished observations and references 15 and 25). To produce skin infection experimentally, the corneal layer must be circumvented by subcorneal injection or the epidermis must be abraded prior to inoculation with bacteria (1, 2, 11-13, 15, 17, 22, 25, 27, 28). In impetigo, infection is localized to the subcorneal layer of the skin, suggesting that once access to differentiated subcorneal keratinocytes is gained, *S. pyogenes* may bind to injured keratinocytes to initiate infection. We have shown previously that differentiation of cultured human keratinocytes in vitro promotes the adherence of *S. pyogenes* (14).

Keratinocytes are injured acutely by UVB light and by bacterial lipopolysaccharide (LPS), leading to increased synthesis of tumor necrosis factor alpha (TNF- α) and interleukin (IL)-1 α and the leakage of these cytokines into the extracellular environment through a damaged plasma membrane (3-5, 19-21, 23, 24, 31, 32). Upregulated IL-1 α and TNF- α expression due to chronic epidermal barrier disruption in essential fatty acid-deficient mice leads to a significant increase in colonization of the skin with *S. pyogenes* (10).

We hypothesized that acute keratinocyte injury promotes adherence of *S. pyogenes*, since streptococcal skin infections such as impetigo develop at sites of cutaneous injury and adherence presumably is an initial step in pathogenesis of infection.

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Keratinocytes were injured by UVB light and LPS. Undifferentiated and differentiated keratinocytes (grown in 0.15 and 1.0 mM calcium, respectively) were cultured from neonatal human foreskins as described previously (14). To injure keratinocytes by exposure to UVB light, the keratinocyte growth medium was removed from the tissue culture plates, leaving a thin film (200 μ l per well) which kept cultures moist. Tissue culture plates were placed in a UVC-1000 UV Crosslinker (Hoefer Scientific Instruments, San Francisco, Calif.) fitted with a bank of four Sankyo Denki F15T8 15W UVB bulbs (Ultra Lum Inc., Carson, Calif.) which emitted light predominantly in the 280- to 340-nm range, with peak irradiance at 302 nm. Radiant energy delivered to the keratinocytes was measured with a CDR-2 electronic radiometer (Ultra Lum Inc.). Timed exposures corresponding to fluencies of 50, 100, 300, and 500 mJ/cm² were delivered, fresh complete keratinocyte growth medium was added, and cultures were replaced in a humidified atmosphere at 36.7°C containing 5% CO₂ for 20 h. The supernatant was sampled, and the release of TNF- α by injured keratinocytes was confirmed by a quantitative sandwich enzyme immunoassay (Human TNF- α Quantikine Immunoassay; R & D Systems, Minneapolis, Minn.) (Table 1). The levels of TNF- α (37 to 111 pg/ml) in the extracellular medium exceeded those reported by others following UVB irradiation of keratinocytes (e.g., \approx 20 pg/ml) (21, 31). Since TNF- α levels were, paradoxically, undetectable following treatment with high-fluency UVB light (i.e., 300 mJ/cm²), as has also been reported for IL-1 α (6), keratinocyte injury by exposure to 300-mJ/cm² UVB light, or to LPS in subsequent experiments (Table 2), was confirmed by measuring lactate dehydrogenase (LDH) release into the supernatant by a colorimetric assay (Sigma Diagnostics, St. Louis, Mo.). After exposure of undifferentiated and differentiated keratinocytes to 300-mJ/cm² UVB light, the LDH concentrations in the incubation medium were elevated to 1,538 \pm 240 and 465 \pm 90 Berger-Broida units, respectively.

Keratinocytes also were injured by adding LPS from *Escherichia coli* O111:B4 (Sigma Chemical Co., St. Louis, Mo.) to keratinocyte cultures at final concentrations of 8 to 100 μ g/ml (Table 2). In general, LDH levels rose with increasing time of exposure to LPS. Injury to keratinocytes by LPS also was confirmed by measurement of elevated TNF- α levels in selected wells (data not shown).

Keratinocyte injury did not affect adherence of *S. pyogenes*. We previously described an in vitro human keratinocyte culture system and adherence assay which demonstrated the ad-

TABLE 1. Release of TNF- α from keratinocytes injured by UVB light

Keratinocyte population	UVB fluency (mJ/cm ²)	TNF- α release \pm SD (pg/ml)
Undifferentiated	0	ND ^a
	50	37 \pm 10
	100	55 \pm 9
	300	ND
Differentiated	0	ND
	50	111 \pm 29
	100	80 \pm 32
	300	ND

^a ND, not detectable (limit of detectability, 15 pg/ml).

herence of *S. pyogenes* to keratinocytes in a manner which simulated human impetigo, whereby the bacteria adhered preferentially to terminally differentiated keratinocytes (14). Utilizing these experimental systems, we tested the role of keratinocyte injury in modulating the interaction of *S. pyogenes* with keratinocytes, using strains of *S. pyogenes* (serotype M52, strain 3732 [7]; serotype M60, strain 4500-1s; serotype M49, strain 5569-1s; M-untyped strains ALAB 48 and ALAB 53; and serotype M49, strain CS101 [18]) associated with superficial skin infections (14). We chose to examine adherence of *S. pyogenes* to keratinocytes, as it is presumed to be an initiating step in the pathogenesis of cutaneous infections. Furthermore, since infection in impetigo is confined histopathologically to highly differentiated, upper-epidermal keratinocytes, we hypothesized that injury enhances adherence preferentially to more completely differentiated keratinocytes.

Contrary to our hypothesis, keratinocyte injury by exposure to UVB light did not affect adherence of M52 serotype, strain 3732 *S. pyogenes* to differentiated keratinocytes (Fig. 1); the small effect of UVB light on adherence to undifferentiated keratinocytes is unlikely to be biologically significant. No effect was seen over a UVB light energy range of 50 to 500 mJ/cm², regardless of whether adherence was initiated 5 to 20 h after irradiation (data not shown). Exposure of keratinocytes to 100 μ g of LPS per ml for 2 to 40 h likewise did not affect attachment of impetigo strain 3732 (Fig. 2). This effect was not strain specific, as adherence of five additional skin-associated strains of *S. pyogenes* (M49 serotype strain 5569, M49 serotype strain CS101, M60 serotype strain 4500, ALAB 48, and ALAB 53) was unaffected by pretreatment of differentiated keratinocytes for 16 h with 100 μ g of LPS per ml (data not shown). Regard-

TABLE 2. Release of LDH from keratinocytes injured by LPS

Keratinocyte population	LPS exposure (h)	LDH release (Berger-Broida units/ml) \pm SD
Undifferentiated	0	412 \pm 159
	2	512 \pm 152
	4	578 \pm 36
	6	768 \pm 103
	12	732 \pm 58
	24	368 \pm 110
	Differentiated	0
2		163 \pm 136
4		275 \pm 49
6		305 \pm 42
12		480 \pm 57
24		820 \pm 78

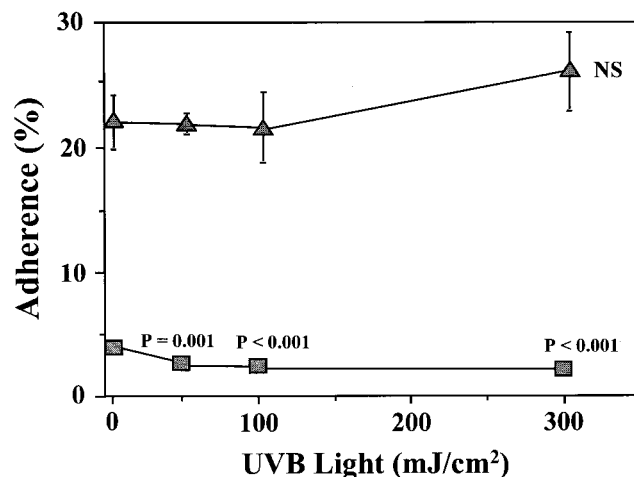


FIG. 1. Injury of differentiated (Δ) or undifferentiated (\square) keratinocytes by UVB light did not affect adherence of *S. pyogenes* (strain 3732). Adherence is expressed as the percentage of the total counts per minute of radiolabelled bacteria added to each well that remained after nonadherent bacteria were washed and vortexed away. Error bars represent standard deviations. NS, non-significant analysis of variance (ANOVA) treatment effect ($F > 0.05$). P values were calculated if the ANOVA for treatment effect was significant and are for comparison with values for the untreated control keratinocytes of the same differentiation state by Student's t test.

less of the injurious stimulus, and also in the absence of injury, adherence was four- to fivefold greater to differentiated than to undifferentiated keratinocytes (Fig. 1 and 2), as we reported previously (14). The variability in percentages of adherence from experiment to experiment reflects differences in the bacterial inoculum, as well as in the propensity of bacteria to bind to keratinocytes from different individuals.

Our data suggest that factors other than acute injury to keratinocytes are important in facilitating infection. Perhaps injury *in vivo* promotes streptococcal adherence via alterations in the epidermis which were not modeled by our experimental system. Injury to corneocytes and disruption of epidermal barrier integrity, rather than keratinocyte injury per se, may be of

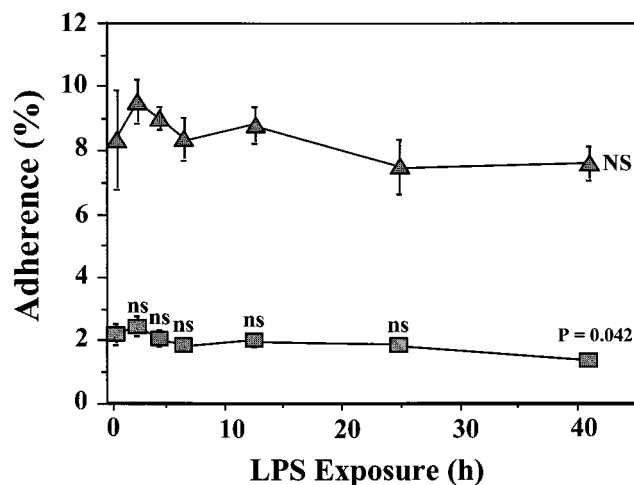


FIG. 2. Injury of differentiated (Δ) or undifferentiated (\square) keratinocytes by LPS did not affect adherence of *S. pyogenes* (strain 3732). Adherence is expressed and data are analyzed as described in the legend to Fig. 1. NS, non-significant ANOVA treatment effect ($F > 0.05$); ns, non-significant difference from the mean of the untreated control as determined by Student's t test.

overriding importance in susceptibility to cutaneous infections. However, the impact of the epidermal barrier on susceptibility to infection could not be assessed with our model system, since the keratinocytes were unable to fully differentiate or form an effective barrier under the experimental conditions.

Previously we reported, and now confirm, that terminal differentiation of keratinocytes promotes the adherence of *S. pyogenes* (14), perhaps through upregulated expression of keratinocyte receptors for binding. Initiation of infection may depend on disruption of the corneal layer, allowing access of bacteria to differentiated, upper-spinous-layer keratinocytes. Ready binding of *S. pyogenes* to keratinocytes (14) is consistent with the hypothesis that adherence to keratinocytes is an important initiating step in the pathogenesis of skin infections (14). Perhaps a factor of principal importance in the host-pathogen interaction which favors the initiation of streptococcal skin infections is the proper modulation of bacterial virulence factors (e.g., adhesins and hyaluronic acid capsule [29]) at the time of contact of the bacteria with subcorneal receptors for adherence. This is the subject of ongoing investigations in our laboratory.

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