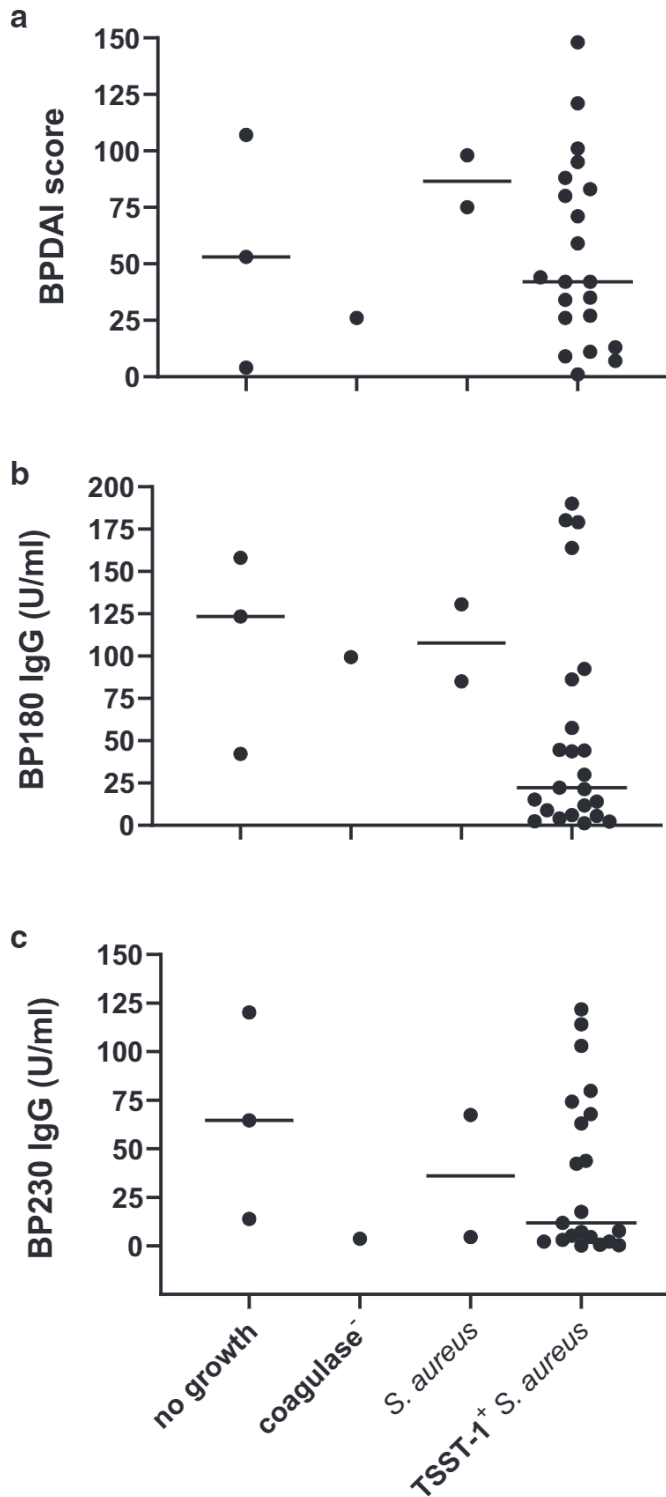


SUPPLEMENTARY MATERIALS AND METHODS

Enrollment of healthy controls for assessment of *Staphylococcus aureus* colonization and staphylococcal toxic shock syndrome toxin-1 IgG titers as a function of age

Healthy individuals on routine visits to the Department of Dermatology at the University of Iowa Hospitals & Clinics (Iowa City), primarily for skin checks and evaluation of benign lesions not known to affect the skin flora, were offered enrollment (Institutional Review Board number 200701758). Patients with any history of autoimmune disease or treatment with antibiotics or immunosuppressive medications in the 2 months before were excluded. Written informed consent was obtained from 168 individuals aged 40–88 years, peripheral blood was collected, and

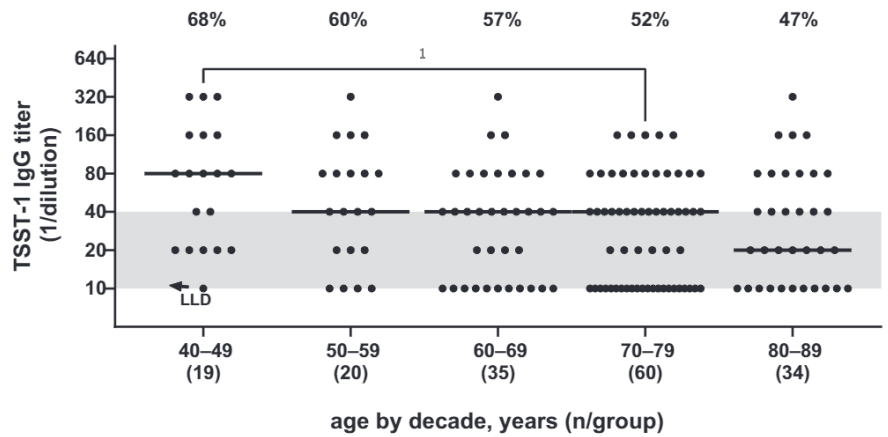
demographic information was recorded. The number of individuals in each age group are as follows (mean age \pm SD, age range, sex): ages 40–49 years, a total of 19 (45.9 \pm 3.2, range = 40.7–49.9, 13 female/6 male); ages 50–59 years, a total of 20 (55.2 \pm 3.1, range = 50.5–59.9, 12 female/8 male); ages 60–69 years, a total of 35 (65.5 \pm 3.1, range = 60.2–69.9, 14 female/21 male); ages 70–79 years, a total of 60 (74.6 \pm 2.7, range = 70.3–79.1, 24 female/36 male); and ages 80–89 years, a total of 33 (83.9 \pm 2.8, range = 80.1–88.4, 17 female/16 male). A Wilcoxon rank-sum test revealed no correlation between toxic shock syndrome toxin-1 IgG titer and sex ($P < 0.05$). Bacterial swabs were also obtained from a subset ($n = 125$, 66 female, 59 male) of these patients.



Supplementary Figure S1. Disease activity and autoantibody levels of patients with BP on the basis of lesional isolate. Staphylococcal colonization of 28 BP lesions was characterized as no growth (n = 3) or growth of coagulase-negative staphylococci (n = 1), *S. aureus* (n = 2), or TSST-1⁺ *S. aureus* (n = 22). Disease activity was scored using the BPDAL, and circulating cutaneous autoantibodies, (b) BP180 and (c) BP230 IgG, were determined by ELISA. Wilcoxon rank-sum test did not reveal any significant differences in BPDAL or BP180/BP230 antibody levels on the basis of the type of bacterial colonization. BP, bullous pemphigoid; BPDAL, Bullous Pemphigoid Disease Area Index; TSST-1, toxic shock syndrome toxin-1.

Supplementary Figure S2. TSST-1 antibody levels decline with age.

TSST-1 IgG titers were evaluated as a function of age in 168 controls aged 40–89 years. When stratified by decades of age (40–49 years, 50–59 years, and others), the frequency of individuals with protective antibody levels, % indicated above group, declined gradually so that 68% (27/40) of subjects aged 40–49 years had titers ≥ 40 , whereas only 47% (16/34) of subjects aged 80–89 years had protective titers (shaded area = nonprotective titers). * $P = 0.0195$ (adjusted) using Kruskal–Wallis test followed by Dunn’s multiple comparisons test for the group aged 40–49 years versus the group aged 70–79 years. Each point represents the average of triplicate wells for each individual, and the horizontal line represents the median value for that group. LLD, lower limit of detection; TSST-1, toxic shock syndrome toxin-1.



Supplementary Table S1. Demographic, Clinical, and Laboratory Information for Patients with BP and the Matched Controls

ID No.	Sex	Age	Medications ³ Previous 2 wk (Dose/d)	Staphylococcal Isolate ²				Controls ¹			
				Lesion (TSST-1 µg/ml) ⁴	Unaffected Skin	Nares	TSST-1 IgG ⁵	Skin	Nares	TSST-1 IgG	
722	F	74.2	Vancomycin 3.5 g, IV	None	ND	ND	40	None	Coagulase negative	Coagulase negative	10
767	F	74.6	Minocycline 200 mg	None	None	TSST-1 ⁺	40	Coagulase negative	Coagulase negative	Coagulase negative	10
723	M	83.4	2 DS sulfamethoxazole/trimethoprim tablets, prednisone taper	None (0.709)	ND	ND	20	Coagulase negative	Coagulase negative	Coagulase negative	80
800	M	78.2	Minocycline 200 mg	Coagulase negative	Coagulase negative	Coagulase negative	20	<i>S. aureus</i>	<i>S. aureus</i>	<i>S. aureus</i>	40
773	F	70.8	Vancomycin 2 gm, IV	<i>S. aureus</i>	ND	ND	10	None	None	None	160
751	F	72.0	Minocycline 200 mg	Coagulase negative <i>S. aureus</i> (4,19)	Coagulase negative TSST-1 ⁺	Coagulase negative <i>S. aureus</i>	40	Coagulase negative	Coagulase negative	Coagulase negative	10
781	F	61.5	None	TSST-1 ⁺	ND	TSST-1 ⁺	160	TSST-1 ⁺	None	None	10
829	M	65.7	Doxycycline 200 mg	TSST-1 ⁺	TSST-1 ⁺	TSST-1 ⁺	80	Coagulase negative	Coagulase negative	Coagulase negative	20
849	F	65.8	None	TSST-1 ⁺	Coagulase negative	<i>S. aureus</i>	<10	None	TSST-1 ⁺	TSST-1 ⁺	20
710	M	66.3	Prednisone 30mg	TSST-1 ⁺	ND	ND	20	TSST-1 ⁺	Coagulase negative	Coagulase negative	160
758	M	67.2	Prednisone 60 mg Mycophenolate 3 g	TSST-1 ⁺ (0.0678)	ND	ND	20	None	Coagulase negative	Coagulase negative	<10
728	M	67.4	None	TSST-1 ⁺ (0.707)	ND	ND	640	Coagulase negative	Coagulase negative	Coagulase negative	40
752 ⁶	M	68	Prednisone taper	TSST-1 ⁺	ND	ND	40	Coagulase negative	Coagulase negative	Coagulase negative	20
820	M	72.6	None	TSST-1 ⁺ (0.163)	ND	TSST-1 ⁺	40	Coagulase negative	Coagulase negative	Coagulase negative	40
568	F	73.0	Prednisone taper	TSST-1 ⁺	ND	TSST-1 ⁺	20	None	Coagulase negative	Coagulase negative	160
807	M	74.3	None	TSST-1 ⁺ (0.066)	Coagulase negative	Coagulase negative	20	None	Coagulase negative	Coagulase negative	40
802	M	74.6	Prednisone 20 mg Mycophenolate 2 g Doxycycline 200 mg	TSST-1 ⁺	ND	Coagulase negative	80	None	Coagulase negative	Coagulase negative	160
811	F	75.3	None	TSST-1 ⁺	Coagulase negative	None	10	Coagulase negative	Coagulase negative	Coagulase negative	<10
712	M	78.7	None	TSST-1 ⁺	ND	ND	20	None	Coagulase negative	Coagulase negative	20
808	M	78.8	None	TSST-1 ⁺	Coagulase negative	Coagulase negative	80	None	Coagulase-negative	Coagulase-negative	20
731	F	81.6	None	TSST-1 ⁺ (0.0075)	ND	ND	20	Coagulase negative	<i>S. aureus</i>	Coagulase negative	0
688	M	84.9	None	TSST-1 ⁺	Coagulase negative	Coagulase negative	40	Coagulase ⁻	None	None	320
864	M	85.6	Prednisone taper	TSST-1 ⁺	TSST-1 ⁺	TSST-1 ⁺	<10	Coagulase negative	Coagulase negative	Coagulase negative	320

(continued)

Supplementary Table S1. Continued
 New-Onset BP

ID No.	Sex	Age	Medications ³ Previous 2 wk (Dose/d)	Staphylococcal Isolate ²				Controls ¹			
				Lesion (TSST-1 µg/ml) ⁴	Unaffected Skin	Nares	TSST-1 IgG ⁵	Skin	Nares	TSST-1 IgG	
810	M	86.3	None	TSST-1 ⁺ (0.053)	None	<i>S. aureus</i>	80	None	Coagulase negative	10	
866	F	86.5	None	TSST-1 ⁺	ND	TSST-1 ⁺	40	Coagulase negative	Coagulase negative	20	
703	F	87.1	None	TSST-1 ⁺ (19.4)	ND	ND	160	Coagulase negative	Coagulase negative	40	
774	M	90.8	None	TSST-1 ⁺	ND	ND	160	Coagulase negative	Coagulase negative	160	
726	M	91.1	None	TSST-1 ⁺	ND	Coagulase negative	40	None	Coagulase negative	80	

Abbreviations: DS, double strength; F, female; ID, identification; IV, intravenous; M, male; ND, not done; No., number; TSST-1, toxic shock syndrome toxin-1.

¹Individual controls were matched to patients with BP by sex and age (±1 year); 16 of 28 (57%) of the controls, and 13 of 28 (46%) had type II diabetes mellitus.

²Staphylococcal isolates were identified by growth on blood agar, and catalase and coagulase tests were used to confirm *S. aureus*; coagulase-negative staphylococci (shaded orange) or no staphylococcal growth (none).

³Medications (duration in weeks/drug or mg/day prednisone) prescribed by a referring physician in the 2 weeks preceding enrollment. Cells shaded in gray indicate antibiotics with staphylococcal coverage.

⁴*S. aureus* was categorized as TSST-1⁺ (shaded blue) or non-TSST-1 (shaded green) by immunoblot, and TSST-1 was quantitated in blister fluids of adequate volume using a standard curve consisting of 10-fold dilutions (10–0.001 µg/ml) of purified TSST-1.

⁵TSST-1 IgG antibody titers were determined by ELISA (lower limit of detection < 10). Titers ≥40 are considered protective.

⁶Subject was of Asian descent. All other subjects were Caucasian.

Supplementary Table S2. BP Lesions Are Highly Colonized with TSST-1⁺ *Staphylococcus aureus*

Sample Type	<i>S. aureus</i> ¹ (% TSST-1 ⁺)	Coagulase-Negative Staphylococci	No Growth
BP ²			
Lesion, n = 28	24, 85.7% ³ (22, 92%) ⁴	1, 3.6%	3, 10.7%
Unaffected skin, n = 11	3, 27.3% (3, 100%)	6, 54.5%	2, 18.2%
Nares, n = 17	10, 58.8% (7, 70%)	6, 35.3%	1, 5.9%
Control			
Skin n = 28	3, 10.7% (1, 33%)	14, 50%	11, 39.3%
Nares n = 28	3, 10.7% (1, 33%)	22, 78.6%	3, 10.7%

Abbreviations: BP, bullous pemphigoid; TSST-1, toxic shock syndrome toxin-1.

¹Growth of gram-positive cocci on blood agar was confirmed as catalase-positive staphylococci and then categorized as coagulase-positive *S. aureus* or coagulase-negative staphylococci.

²Swabs were obtained from patients with new-onset BP and sex- and age-matched controls.

³Data are expressed as the number and percentage of total samples obtained at each location.

⁴TSST-1 was detected by immunoblot of isolated colonies, and the numbers in parenthesis indicate the number and percentage of TSST-1⁺ of the total number of samples that grew *S. aureus*.