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Strains of *Streptococcus pyogenes* from Severe Invasive Infections Bind HEp2 and HaCaT Cells More Avidly than Strains from Uncomplicated Infections

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Epidemiologically unrelated *Streptococcus pyogenes* strains isolated from blood, throat, and skin were assayed for adherence to HEp2 and HaCaT cells. Invasive isolates showed significantly higher avidity for these cell lines than isolates from skin and throat. In general, *S. pyogenes* showed greater binding to HaCaT cells than to HEp2 cells.

Streptococcus pyogenes (group A streptococcus [GAS]) is an etiological agent for diverse human diseases, including pharyngitis, pyoderma, and severe invasive diseases. In addition, the pathogen is associated with potentially life-threatening sequelae such as poststreptococcal glomerulonephritis and acute rheumatic fever. In the Northern Territory (NT) of Australia the incidence of acute rheumatic fever is very high among the indigenous population (3), despite a low throat isolation rate of GAS. Furthermore, pyoderma from GAS infection is extremely common and poststreptococcal glomerulonephritis is endemic in many remote Aboriginal communities (4, 7). While asymptomatic throat carriage is often the reported reservoir for strains associated with invasive disease (5), in populations where impetigo is endemic, such as in Aboriginal communities in the NT, the primary reservoir is the skin. Irrespective of which tissue is the primary site of infection, the first event the pathogen needs to achieve is adherence to host cells. The *S. pyogenes* genome encodes numerous genes that could be regarded as encoding adhesins. These genes are highly regulated, and individual strains do not have the genetic potential to encode all of these proteins. The adhesins include M protein (an antiphagocytic molecule), capsule, and fibronectin binding proteins. There are many different fibronectin binding proteins, such as SfbI (8, 12), PrtF2 (9, 10), Fbp54 (2), and SfbII (11). The adherence capacity of an individual strain could vary depending on the repertoire of genes for the adhesins that the strain possesses and their level of expression. This in turn may reflect the differences in the ability to colonize and persistently infect different tissue sites. A corollary of this is that isolates from different tissue sites may exhibit differences in adherence capacity. To test this, we have determined the extent of binding of GAS isolates from skin, throat, and blood to HEp2 and HaCaT cell lines, representing human laryngeal epithelial cells and keratinocytes, respectively.

GAS isolates from the NT were collected between 1990 and 2002. The 72 strains analyzed in this study were isolated from blood ($n = 26$), skin ($n = 22$), and throat ($n = 24$) (Table 1). Blood isolates were from severe disease, and the remaining strains were from uncomplicated infections. The isolates were Vir typed as described previously (6). Vir typing involves restriction fragment length polymorphism of the *mga* regulon, which includes the gene for highly variable M protein. To ensure inclusion of epidemiologically unrelated strains, one representative isolate from each Vir type was included. Cultures were grown overnight at 37°C in an orbital shaker to stationary phase in Todd-Hewitt broth (Oxoid, Basingstoke, United Kingdom) supplemented with 1% yeast extract. To prepare the GAS inoculum for adherence assays, overnight cultures were centrifuged, and the pellets were washed in phosphate-buffered saline (PBS; Life Technologies Gibco BRL, New York, N.Y.) and resuspended in serum-free and antibiotic-free RPMI 1640 medium (Life Technologies) to an optical density at 600 nm of 0.05. This represents approximately 1×10^7 to 1.5×10^7 bacteria per ml.

Human laryngeal epithelial (HEp2) cells were maintained in RPMI 1640 medium supplemented with 10% fetal calf serum (Life Technologies), 1% Fungizone (Life Technologies), 20 μg of vancomycin HCl (David Bull Laboratories, Sydney, Australia) per ml, and 100 μg of streptomycin sulfate (Sigma, St. Louis, Mo.) per ml. Human adult skin keratinocytes (HaCaT cells) were maintained in Dulbecco's modified Eagle medium (Life Technologies) supplemented with 10% heat-inactivated fetal calf serum. For adherence assays, $\sim 10^5$ cells/ml were seeded onto 12-mm-diameter glass coverslips in the bottoms of 24-well tissue culture plates (Nunc, Roskilde, Denmark). After overnight growth at 37°C in 5% CO_2 atmosphere, the cells were washed with PBS (pH 7.4) and inoculated with 500 μl of the GAS inoculum. After 2 h of incubation at 37°C, the coverslips were washed five times by adding 1 ml of PBS to each well, and after gentle mixing, the wash solution was removed by aspiration. After removal of the nonadherent bacteria, the host cells and adherent bacteria were fixed with 95% methanol and air-dried. After heat fixing, the coverslips were placed on slides and Gram stained for viewing under oil immersion. In each

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TABLE 1. Vir type, binding, and source of isolation of GAS isolates used in this study

Isolate	Source[a]	VT[b]	Binding to[c]:	
			HEp2	HaCaT
NS1	B	23	222	104.5 ± 37.5
NS6	B	12.2	151.5 ± 14.5	183
NS13	B	24	114.5 ± 108	385 ± 12
NS20	B	3.4	127.5 ± 6.5	298 ± 73
NS25	B	18	86.5 ± 0.5	204 ± 6
NS27	S	4	283 ± 16	361.5 ± 5.5
NS43	B	9	131.5 ± 2.5	250.5 ± 56.5
NS50	B	5	66.5 ± 5.5	120 ± 38
NS53	S	29.1	146.5 ± 1.5	257.5 ± 0.5
NS66	B	41.2	161.5 ± 11.5	193 ± 7
NS78	B	30	88.5 ± 25.5	202.5 ± 4.5
NS80	B	69	310	267.5 ± 59.07
NS101	B	33.1	57.5 ± 12.5	
NS125	B	20.1		303.5 ± 34.53
NS178	B	26	172.5 ± 8.5	465.5 ± 10.5
NS180	T	110	0	2.0 ± 2.0
NS182	T	60	314	145.75
NS195	B	120	253	401 ± 22
NS204	B	121	34.5 ± 0.5	206 ± 16
NS205	B	20.2	108.5 ± 3.5	152 ± 56
NS223	B	4	166 ± 11	411 ± 5
NS225	B	115	174.5 ± 25.5	515.33
NS226	B	14.1	293	210 ± 41.34
NS234	T	42.1	175 ± 14	226 ± 28
NS235	B	118	173 ± 10	207 ± 15
NS236	T	111	45 ± 1	231.75
NS265	S	11	73 ± 11	181 ± 51
NS344	B	127	97 ± 4	
NS351	S	22	108	
NS436	S	3.3	92 ± 31	152
NS476	B	39	253.5 ± 5.5	329 ± 13
NS506	S	134		216.5 ± 1.5
NS564	S	119	117.5 ± 21.5	250 ± 5
NS567	B	38	131.5 ± 0.5	333.5 ± 9.5
NS581	S	42	57 ± 3	108.5 ± 2.5
NS611	S	3.7	85 ± 11	135.5 ± 1.5
NS671	S	133	136.5 ± 2.5	62 ± 6
NS678	T	113	163.5 ± 39.5	84
NS696	T	114	28 ± 11	
NS803	S	131	40 ± 1	127 ± 10
NS804	S	130	82 ± 16	188.5 ± 35.5
NS880	T	52	32 ± 8	267.5 ± 16.5
NS931	B	57	68.5 ± 6.5	369 ± 98
NS980	S	129	132.5 ± 15.5	50.5 ± 3.5
NS1030	S	77	94.5 ± 19.5	112.5 ± 10.5
NS1033	S	3.22	109.5 ± 11.5	68.5 ± 4.5
NS1045	S	117	50.5 ± 3.5	54.5 ± 10.5
NS1095	S	128	88.5 ± 58.5	251.5 ± 56.5
NS1096	S	32	40 ± 1	122 ± 34
NS1099	S	126	83 ± 19	125 ± 10
NS1107	T	33.3	285.5 ± 13.5	159.5 ± 18.5
NS1120	T	105	134 ± 40	164
NS1122	S	65	74	129 ± 1
NS1133	B	17.1	56.5 ± 10.5	259 ± 5
NS1140	B	101	147 ± 5	122 ± 66.52
NS1210	S	125	230 ± 4	522
NS1215	T			316.5 ± 6.5
NS1216	S	49.1	220	81 ± 3
NS1350	T	17.5	70.5 ± 8.14	299 ± 3
3A	T	14.3	50.5 ± 14.33	50 ± 36.8
11A	T	New	10.75 ± 1.09	328.5 ± 40.21
24A	T	New	99.83	228 ± 78.89
30A	T	3.6	130.5 ± 0.5	329.33 ± 51.55
51A	T	New	202 ± 23.39	
52A	T	3.24	27.33 ± 7.04	57.75 ± 14.79
66A	T	37.1	21.5 ± 2.87	55.5 ± 8.08
95A	T	3.41	12.5 ± 2.5	9.0
100A	T	3.23	9 ± 1	68 ± 13.44
101A	T	39.1	102 ± 6	262.17 ± 41.55
244op	T	New	33.75 ± 12.5	186.5 ± 28.66
CK232	T	3.5	119 ± 14	261.5 ± 11.5
CK410	T	28	73	184 ± 7.0
Avg			117 ± 78.6	208 ± 119

[a] S, skin; B, blood; T, throat.
[b] VT, Vir type.
[c] Number of GAS bound per 50 HEp2 or HaCaT cells (mean ± standard deviation).

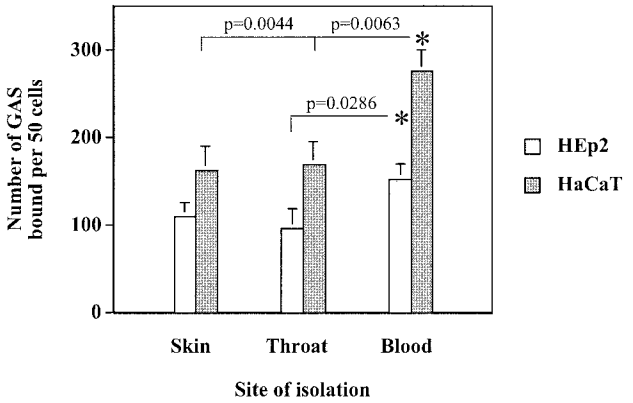

FIG. 1. Comparison of binding of GAS to HaCaT and HEp2 cell lines. Statistically significant results are indicated by asterisks. Error bars represent standard errors.

experiment, cells in several random fields were analyzed, and attachment was expressed as the average number of GAS chains per cell. All assays were performed in duplicate, and the mean binding was determined for each strain. All statistical analyses were performed with Stata Statistics/Data Analysis program version 7.0 (Stata Corporation, College Station, Tex.). Data were analyzed with *t* tests.

GAS strains adhered to both cell types, and the degree of binding varied from strain to strain (Table 1). There was good correlation between independent experiments with 20 isolates repeated at two time intervals (data not shown), suggesting the avidity of binding is reproducible and strain specific. Overall, GAS binding to HaCaT cells is greater than to HEp2 cells ($P < 0.05$). When the data in Table 1 were separated based on the tissue site of isolation, an average of 270 chains of GAS strains from blood bound to 50 HaCaT cells (Fig. 1). In contrast, skin and throat isolates bound on average only 169 and 178 chains per 50 HaCaT cells, respectively. These differences are statistically significant ($P = 0.0044$ and 0.0063, respectively). Interestingly, for HEp2 cells the differences are less pronounced and not statistically significant. However, when the data were reanalyzed based on invasive versus uncomplicated infections by combining data for the skin and throat isolates, significant differences between the two categories were found in both cell lines ($P = 0.0011$ for HaCaT; $P = 0.0238$ for HEp2).

Earlier work from this laboratory showed that many commonly circulating strains of *S. pyogenes* could cause invasive disease with skin as the primary site of infection (1). These observations are consistent with the present findings of higher avidity of the NT GAS strains for HaCaT than HEp2 cell lines and blood isolates being able to bind in greater numbers than the isolates from uncomplicated infections. Possible explanations for the high adherence propensity of *S. pyogenes* blood isolates include both genotypic and phenotypic differences between isolates from invasive and noninvasive disease sources. Further studies are required to define the nature of this binding avidity and to determine whether it is consistent within clonal populations.

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