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agr-Dependent Bacterial Interference Has No Impact on Long-Term Colonization of *Staphylococcus aureus* during Persistent Airway Infection of Cystic Fibrosis Patients

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The *agr* specificity group distribution of persistent *Staphylococcus aureus* clones recovered from the airways of cystic fibrosis (CF) patients did not differ from that of isolates recovered from various clinical infections and healthy nasal carriers. The success of CF clones in terms of cocolonization and/or infection with *S. aureus*, prevalence of clones, or persistence appeared to be independent of *agr* group specificity.

In cystic fibrosis (CF), *Staphylococcus aureus* is highly prevalent and the course of colonization and infection is often persistent in spite of antistaphylococcal therapy (1, 2, 7, 15). The *agr* system of *S. aureus* represents a quorum sensing system. A secreted autoinducing peptide induces activation of the *agr* operon at a given threshold, thereby up-regulating extracellular protein production, while down-regulating the synthesis of cell wall-associated proteins, which serve as adhesins for the pathogen to host tissues (9). Sequence variability of the autoinducing peptide and its transmembrane receptor *agrC* allows the distinction of four *agr* specificity groups (4). While a member of a given *agr* group activates the *agr* response of a strain of the same group, strains of different *agr* groups cause the inhibition of the *agr* response. This type of bacterial interference has been suggested to influence colonization dynamics by enhancing or inhibiting the ability of *S. aureus* to colonize in the presence of resident strains (6). Furthermore, an association between *agr* groups and strains causing certain diseases has been described: *agr* group III has been associated with toxic shock syndrome (13), and *agr* group IV has been associated with staphylococcal scalded skin syndrome (4, 5). However, there is no knowledge about the association of a specific *agr* group and CF airway infection or about *agr*-dependent bacterial interference in persistent *S. aureus* colonization and/or infection in CF patients.

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The CF isolates used in the present study were collected during a 6-year prospective study from 50 CF patients (8). Molecular typing by pulsed-field gel electrophoresis (PFGE) of 685 *S. aureus* isolates allowed the distinction of 45 different *S. aureus* clones. Thirty-eight individual clones (isolates with distinct fragment patterns by PFGE analysis) were isolated from single patients, while six prevalent clonal lineages (38 isolates with indistinguishable fragment patterns or with fewer than 7 fragment differences) were cultured from more than two patients (17). An infection was considered persistent if isolation of *S. aureus* continued for more than 6 months. Thirty-three of 50 (66%) patients were persistently infected by a single *S. aureus* clone, while 17 (34%) patients were infected by several clones. For each patient, one isolate of every different clone was subjected to multiplex PCR for *agr* group determination (10).

Sixty-four *S. aureus* isolates were collected from patients with various *S. aureus* infections. Two hundred nineteen isolates from patients with bacteremia, collected during a German multicenter study, were included (18). As a control, 88 nasal carriage isolates were cultured from healthy volunteers from the same geographic area as the CF patients.

The multiplex PCR for *agr* group determination (10) revealed that the pattern of *agr* distribution did not differ between the three different clinical settings and the control group, with *agr* group I strains being the most prevalent strains (Table 1). These findings seem to reflect the natural distribution of *S. aureus* lineages in this geographical area according to *agr* groups and are similar to findings of other studies (3, 14, 16).

The mean persistence durations of *agr* group I, II, and III were similar (28 months); they differed from the mean persistence of *agr* group IV clones (23.5 months), which were isolated from only four patients. Thus, the persistence of *S. aureus* for our study isolates did not vary significantly among *agr* groups, indicating that the clones of the different *agr* types were equally fit in persistence.

The prevalence of *agr* groups for patients infected by a single *S. aureus* clone (*agr* group I, 45.7%; *agr* group II, 25.7%; *agr* group III, 20%; *agr* group IV, 8.5%) did not differ from the prevalence for patients infected by several clones (*agr* group I, 44.2%; *agr* group II, 30.2%; *agr* group III, 23.2%; *agr* group IV, 2.3%), suggesting that no clone of a particular *agr* group was more competent than another in inhibiting colonization by other clones.

Seventeen CF patients (34%) were infected by several *S. aureus* clones. Most patients (15 of 17) harbored clones that belonged to different *agr* groups (Table 2). In clones with different *agr* groups, the *agr* signaling pathway may be inhibited, leading to down-regulation of secreted proteins and up-

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TABLE 1. Prevalence of *agr* groups in different clinical settings

agr group	No. (%) of isolates from:				Total
	CF patients	Various infection sites[a]	Multicenter study[b]	Nasal carriage	
I	34 (44)	29 (46)	96 (44)	41 (47)	200 (45)
II	22 (29)	16 (24)	63 (29)	21 (24)	122 (27)
III	17 (22)	12 (19)	50 (23)	25 (28)	104 (23)
IV	4 (5)	7 (11)	9 (4)	1 (1)	21 (5)
Total	77 (100)	64 (100)	218 (100)	88 (100)	447 (100)

[a] Clinical isolates were recovered from patients with endocarditis (n = 7), osteomyelitis (n = 8), skin and soft tissue infections (impetigo, bursitis, staphylococcal scalded skin syndrome, finger pulp infection, cellulites, abscesses, and wound infection [n = 25]), device-related infection (n = 15), chronic otitis media (n = 7), pneumonia (n = 1), and cerebral nervous system infection (n = 1).

[b] Bacteremic isolates.

regulation of adhesive proteins. Such enhanced expression of adhesive proteins, e.g., fibronectin binding protein, would support the adhesion to host tissues, and in the case of CF, adhesion to the upper and lower airway epithelium (11, 12). Therefore, *agr*-related bacterial interference appears to affect early cocolonization of strains by supporting colonization of strains with different *agr* groups.

For 13 of 17 patients colonized and/or infected by several *S. aureus* clones, competition of clones for colonization was observed (Table 2). The success of a clone was suggested if a new clone was able to replace a resident clone and vice versa. Replacement of a clone was considered to have occurred if the respective clone was not isolated for 1 year or longer. In the case of cultures that were negative for at least 1 year before recovery of a new *S. aureus* clone, we assumed that no competition of clones occurred. If the clones were cultured in parallel or consecutively without loss of a clone, no success of a single clone was observed. Thus, in our study no clone of a particular *agr* group was more successful than another (Table 2). Interestingly, we observed that several clones of the same *agr* group as well as of different *agr* groups replaced another clone. Replacement of clones of the same *agr* group is consistent with the concept of *agr*-related bacterial interference (6). By triggering each other's *agr* response, the expression of adhesive proteins is inhibited, which presumably is deleterious for adhesion, and therefore for colonization. However, the finding that clones with different *agr* groups succeeded over other clones in terms of colonization for extended periods implies that mechanisms other than *agr*-related bacterial interference were more important for the long-term success of the clone in the host. An example for the replacement of a clone belonging to *agr* group I by a clone belonging to *agr* group II is given in Fig. 1.

Thirty-nine patients were colonized and/or infected by one of six prevalent clonal lineages (Table 3). A significant association of prevalent clonal lineage and *agr* group was found for clone B, *agr* group I (χ^2 = 6.42; P = 0.0104), isolated from 10 patients, and for clone A, *agr* group III (χ^2 = 7.12; P = 0.008), recovered from 12 patients. However, because these two prev-

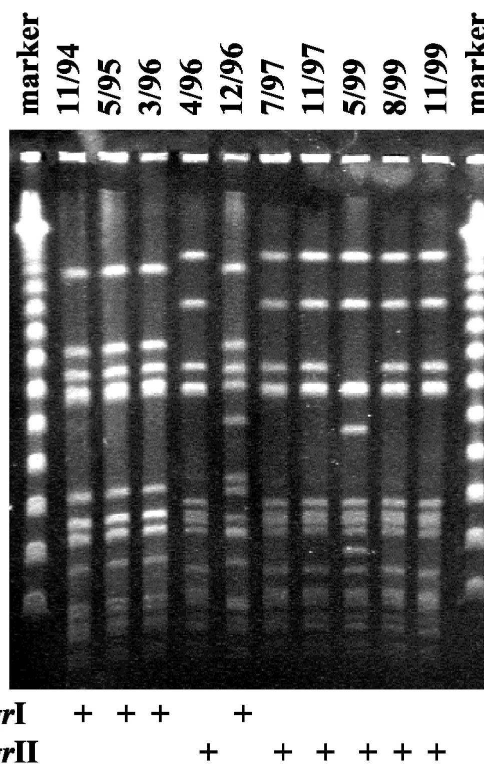

FIG. 1. Replacement of an *agr* group I clone by an *agr* group II clone, as shown by PFGE of consecutive isolates (from the sputum of a CF patient, collected from November 1994 to November 1999) after *Sma*I digestion of chromosomal DNA. The *agr* group I clone was isolated for 17 months before the *agr* group II clone was isolated for the first time in April 1996. Then, the *agr* group I clone was isolated only once again (December 1996), with some differences in fragment patterns, after it was replaced by the *agr* group II clone.

TABLE 2. Analysis of bacterial interference according to *agr* groups of persistent *S. aureus* strains isolated from CF patients

Patient no.	No. of clones[a]	*agr* groups[b]	Success of *agr* group[c]
1	2	II and III	III > II
2	2	I and II	No competition[d]
3	3	I, II, and III	No competition
4	2	I and II	I > II
5	3	I^1, I^2, and III	I^1 > III, I^2 > I^1
6	2	I and II	II > I
7	3	I^1, I^2, and III	I^2 > I^1, I^2 > III
8	2	II and III	III > II
9	3	I^1, I^2, and I^3	No competition
10	3	II, III^1, and III^2	II > III^1, II > III^2
11	3	II^1, II^2, and III	III > II^1, III or II^2
12	2	I^1 and I^2	I^2 > I^1
13	2	I and II	No competition
14	3	I^1, I^2, and II	I^1 > I^2, II > I^2, I^1 > II
15	2	I and III	III > I
16	3	II, III, and IV	No competition
17	3	I^1, I^2, and II	II > I^1, II > I^2

[a] Number of different clones cultured from a single patient as determined by PFGE.

[b] *agr* groups of the different clones from a single patient (with different or the same *agr* groups, as indicated by the superscript number).

[c] *agr* group of a clone that replaced another clone.

[d] The different persistent strains were isolated independently (after a time break of more than 1 year, during which no *S. aureus* strain was isolated or the different strains were cultured in parallel or consecutively without replacement of a strain).

TABLE 3. Prevalence of prevalent clonal lineages versus individual clones within a particular *agr* group

agr group	No. of patients with:		P^a	Total no. of patients (no. of clones)
	Individual clones	Prevalent clonal lineages[b]		
I	17	10 (B)	**0.012**	34 (20)
		4 (D)	0.204	
		3 (E)	0.338	
II	12	7 (C)	0.052	22 (14)
		3 (G)	0.341	
III	5	12 (A)	**0.008**	17 (6)
IV	4	0	ND	4 (4)
Total	38	39 (6)		77 (44)

[a] *P* value comparing the number of individual or prevalent clones of a particular *agr* group to the number of all clones of this specific *agr* group. Boldface values indicate statistical significance.

[b] Capital letters in parentheses indicate distinct clonal lineages. A number in parentheses indicates the number of different prevalent clonal lineages.

alent clones with statistically significant association to a particular *agr* group belonged to different *agr* groups, the prevalence of clones appears to be not affected by *agr* group specificity and *agr* group-associated genes.

In summary, CF-related persistent airway infection was not associated with a distinct *agr* specificity group. While *agr*-related bacterial interference appears to have an impact in early cocolonization of clones, yet-unknown factors other than *agr*-related interference were important for the late success of clones in terms of cocolonization, occurrence of prevalent clones, and persistence in CF airway infection.

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