

Sensitization to aztreonam and cross-reactivity with other beta-lactam antibiotics in high-risk patients with cystic fibrosis

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The immunogenicity, allergenicity, and cross-reactivity of aztreonam were investigated in 21 patients with cystic fibrosis (CF) (aged 5 to 39 years) with well-documented histories of allergic systemic reactions (SRs) to penicillin and/or cephalosporin antipseudomonal β -lactam antibiotics (BLAs). Skin tests (STs) with penicilloyl-polylysine (PPL), penicillin minor determinant mixture, and antipseudomonal BLA were positive in 19 patients (90%). The BLA causing the most recent allergic reaction, minor determinant mixture, or PPL, was positive in 89%, 53%, and 32% of ST-positive patients, respectively. Serum PPL-specific IgE antibodies were not detectable, although PPL-specific IgG antibodies were found in 64% of patients tested. STs to aztreonam reagents were performed and were initially negative in 20 patients. One patient was ST positive to the polylysine conjugate of hydrolyzed aztreonam (SQ 27629), despite no prior exposure to aztreonam, and was not treated. Of 20 patients treated with aztreonam, four were demonstrated to be sensitized by exposure (one had an SR during initial treatment course, two had SRs on reexposure, and one patient was asymptomatic after intravenous desensitization) by positive aztreonam reagent skin responses on repeat testing. Aztreonam-specific IgE and IgG serum antibodies were not detected in any patients, including patients with allergic reactions to aztreonam. Thus, aztreonam is generally well tolerated in high-risk patients with CF allergic to other BLAs and appears to have reduced immunogenicity by serologic testing. However, caution should be exercised with aztreonam in BLA-allergic patients with CF in light of 5% preexisting ST cross-reactivity and 20% sensitization rates found in this study. (J ALLERGY CLIN IMMUNOL 1991;87:78-88.)

PA is responsible for debilitating chronic respiratory tract infection in most patients with CF.¹ Allergic reactions to intravenous BLAs are an important problem for patients with CF because these drugs are an essential component of anti-PA treatment for exacer-

Abbreviations used

BLA:	β -Lactam antibiotic
CF:	Cystic fibrosis
MDM:	Minor determinant mixture (penicillin G, penicilloate and penilloate)
MWD:	Mean wheal diameter
PA:	<i>Pseudomonas aeruginosa</i>
PBS:	Phosphate-buffered saline
PCN G:	Penicillin G
PPL:	Penicilloyl-polylysine
SR:	Systemic reaction
ST:	Skin test

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bations of chronic PA-associated endobronchitis.² Generally, an allergic reaction to a BLA indicates an increased risk for subsequent reactions to the same or other BLAs.^{3,4}

Aztreonam, the first monobactam to be licensed for use in the United States, has excellent in vitro activity against PA and has been effective in early clinical

trials in patients with CF.⁵⁻⁸ Aztreonam shares a β -lactam ring structure with other anti-PA BLAs. It has a large aminothiazole side chain identical to that of the anti-PA BLA, ceftazidime.⁹ Despite these similarities, clinical trials suggest that aztreonam may be less immunogenic than other BLAs, possibly because it lacks a bicyclic nucleus sharing cross-reactivity with the major penicilloyl metabolite of penicillin.^{10, 11} Animal studies have demonstrated weak immunogenicity of aztreonam, noncross-reactivity of IgE antibodies directed against other BLAs with aztreonam, and selective induction of side chain-specific antibodies by aztreonam immunization, suggesting that aztreonam may be suitable for use in patients clinically allergic to other BLAs.¹² Several patients without CF who were allergic to another BLA tolerated aztreonam without desensitization, suggesting that aztreonam may not be cross-reactive in vivo.^{11, 13-15}

The high-dose, repetitive, parenteral exposure of patients with CF to a variety of BLAs and the high rate of allergic SRs in these individuals make this a unique population in which to study issues of BLA immunogenicity, allergenicity, and cross-reactivity.¹⁶ In this study, the immunogenicity, allergenicity, and cross-reactivity of aztreonam in patients with CF and with a history of allergy to other BLAs were investigated with data collected at three institutions.

METHODS

Patient selection

Patients were eligible for study if they had a well-established diagnosis of CF, were at least 4 years of age, had a history of a generalized immediate hypersensitivity reaction to a BLA, and were initially evaluated with an exacerbation of chronic endobronchitis caused by an aztreonam-susceptible strain of *PA*. Pregnant or lactating women were excluded, as were patients with inadequate ST sites, patients with a history of allergy to aztreonam, patients with a history of delayed-onset reactions to BLA without a preceding immediate reaction, or patients treated with an investigational drug within the preceding 30 days. Informed consent was obtained before enrollment. Patients at CF Centers at Stanford University School of Medicine, Louisiana State Medical Center in Shreveport, La., and Eastern Virginia Medical Center, Norfolk, Va., were evaluated. The evaluation and treatment protocol was approved by authorized human subjects review boards at the respective institutions, and informed consent was obtained before enrollment in each patient.

Patients were evaluated on entry by complete history and physical examination. Pertinent history included date of allergic BLA reaction, nature of the illness for which the drug was administered, name, dose and route of BLA, time lapse between initiation of treatment and onset of reaction, detailed description of the reaction and its treatment, and subsequent exposure history and reactions to the same or other

BLAs. Dates of prior treatment with aztreonam, if any, were noted.

BLA skin testing

To assess the cross-reactivity of aztreonam to other BLAs, patients with a history of BLA allergy were skin tested by prick and intradermal application with major and minor determinants of PCN G and with the anti-PA BLA responsible for the most recent allergic reaction (primary drug). Primary drugs included ticarcillin, piperacillin, azlocillin, or ceftazidime. In some instances, other BLAs responsible for additional allergic reactions were also included in the ST panel. Patients were required not to have received opiates, β_2 -adrenergic agonists, or β -blockers within the previous 6 hours, or antihistamines within the previous 3 days (extended to 7 days for hydroxyzine and 14 days for terfenadine) before skin testing. During skin testing, resuscitative equipment and personnel were present, and patients were observed for signs of SRs. If STs were negative, the patient was considered nonreactive and treated with any BLA of the physician's choice. If a patient was ST positive to any BLA, he or she was immediately skin tested to aztreonam reagents, as described below. If a positive ST occurred to an aztreonam reagent, the patient was either not treated or desensitized to a BLA of the attending physician's choice. If aztreonam STs were negative, the patient was treated with intravenous aztreonam (50 mg/kg per dose, maximum, 8 gm/day) for 10 to 21 days.

If a patient with a history of a reaction to a BLA for 1 week or less before the study demonstrated negative ST responses to BLA reagents, STs were repeated 1 month later. Repeat skin testing was performed in such patients because of the possibility of false-negative results during a refractory period in the days after an allergic reaction.

While patients were hospitalized, those patients receiving aztreonam were examined daily, and clinical responses were recorded. Patients were monitored during infusion of the initial dose of aztreonam. Vital signs were recorded at least twice daily. Baseline and weekly laboratory tests included complete blood count (hemoglobin, hematocrit, white count and differential, and platelet count), serum chemistries (glucose, creatinine, urea nitrogen, bilirubin, aspartate aminotransferase, and alanine aminotransferase), and urinalysis. Aztreonam treatment was stopped (1) if an allergic reaction attributable to aztreonam occurred, (2) if there was a failure to respond satisfactorily to treatment within 3 days, (3) if aztreonam-susceptible *PA* was not isolated from pretreatment sputum culture, or (4) if any other adverse event attributable to aztreonam occurred.

STs were performed on the patient's volar forearm by epicutaneous prick testing with solid bifurcated needles. If the results were negative, intradermal injections of 0.02 ml followed. ST responses were read 15 minutes after placement by measuring the largest wheal diameter, the orthogonal diameter, and averaging the two values to produce an MWD. An MWD of ≥ 4 mm with accompanying erythema in the presence of a negative control MWD < 4 mm was selected before the study as the primary criterion for a pos-

TABLE I. Clinical and in vitro allergy evaluation data

Pt	Age/Sex	1° drug	Lapse (mo)	ST + (MWD)	Control (ID MWD)	Comments
1	14/F	Pip	43	None	0	Prior aztreo × two
2	36/M	Ticar	51	Ticar ID 2 mg (7)	1.5	
3	16/M	Pip	48	PPL ID (5.5)	0	Prior aztreo × six
				MDM ID (5)		
				Pip ID 2 mg (5)		
				Ticar ID 2 mg (5)		
4	35/F	Ceftaz	19	MDM ID (9.5)	0	Prior aztreo × three
5	15/M	Ticar	36	PPL ID (5.5)	0	Prior aztreo × one
6	16/M	Azlo	33	Azlo ID 20 µg (5)	0	
7a	11/M	Azlo	43	Azlo ID 2 mg (7.5)	0	
7b				PPL prick		Test b done 17 mo after
				MDM prick		test a
				Azlo prick 20 µg		
8	5/F	Ceftaz	0	Ceftaz ID 2 mg (5)	0	
9	29/F	Ceftaz	6	Ceftaz prick 2 mg		
				MDM ID (7)	1	
10	23/F	Ticar	18	PPL ID (9)		
				MDM ID (7)		
				Ticar ID 20 µg (9)	4	
11a	16/F	Ceftaz	4	Ceftaz prick 2 mg		
11b				SQ 26992 ID (4.5)	1.5	Test b done 9 mo after
						test a with aztreo
						reagents only
12	20/M	Ticar	32	PPL prick		
				MDM prick		
				Ticar ID 20 µg (9)	5.5	
13	26/M	Ceftaz	12	Ceftaz prick 20 µg		
				Pip prick 2 mg		
				MDM ID (9.5)	6.5	
14a	39/F	Ticar	72	MDM prick		
				Ticar ID 2 mg	6	
				(10.5)		
14b				MDM prick		Test b done 9 mo after
				Ticar ID 20 µg (6)	3.5	test a
				Azlo ID 20 µg (6)		
				Ceftaz ID 20 µg (5)		
				Aztreo ID (5)		
				Arginine ID (4.5)		

Pt, Patient; Pip, piperacillin; Ticar, ticarcillin; Ceftaz, ceftazidime; Azlo, azlocillin; Aztreo, aztreonam; ID, intradermal; Tobra, tobramycin.

itive ST.⁴ However, in some subjects, intradermal injections of 0.02 ml of negative control resulted in an MWD of ≥ 4 mm (see below; Table I). In such cases, an MWD ≥ 3 mm larger than the negative control MWD with accompanying erythema was selected as the secondary criterion for a positive ST.¹⁷ For example, if a negative intradermal control MWD on a patient was 5 mm, the primary ST criterion could not be used; in this case, a positive ST was defined as ≥ 8 mm with surrounding erythema.

ST reagents used in the initial BLA battery included (1) PPL (Pre-Pen, lot 11693, Kremers-Urban Co., Milwaukee, Wis.) at 6×10^{-5} mol/L, (2) MDM each at 10^{-2} mol/L, (3) the primary drug at 20 µg/ml, and, if necessary, 2 mg/ml. MDM was prepared at Kremers-Urban Co. (experimental batch X5, lots a to j), as previously described,

and lyophilized for reconstitution before use.¹⁸

The purity of MDM components was documented by high-performance liquid chromatography of one vial from each lot.¹⁹

If any ST to BLA was positive, prick and intradermal testing with aztreonam reagents was then performed. Aztreonam reagents included (1) aztreonam at 6×10^{-3} mol/L in 0.21% L-arginine solvent, (2) arginine solvent alone, (3) SQ 26992, the open-ring hydrolysis product of aztreonam at 6×10^{-3} mol/L, and (4) SQ 27629, the polylysine conjugate of SQ 26992 at 6×10^{-3} mol/L (Fig. 1). Aztreonam reagents were prepared at the Squibb Institute for Medical Research, Princeton, N.J., as previously described, and lyophilized for reconstitution before use.¹¹

Reagents were reconstituted in sterile physiologic PBS,

TABLE I. Cont'd

Pt	Age/Sex	1 ^o drug	Lapse (mo)	ST + (MWD)	Control (ID MWD)	Comments
15	25/M	Azlo	15	MDM prick PPL ID (7) Azlo ID 20 µg (7) Ceftaz ID 20 µg (8)	4	Prior aztreo × one
16	29/M	Pip	65	Pip ID 2 mg (8.5)	5.5	
17a	20/F	Pip	31	Pip prick 2 mg SQ 27629 ID (9.5)	6.5	No prior exposure to aztreo
17b				SQ 27629 ID (9)	5	Test b done 2½ mo after test a with aztreo reagents only
18	24/F	Ceftaz	24	MDM prick Ceftaz prick 20 µg		
19a	29/F	Pip	65	None	6.75	Prior aztreo × one
19b				SQ 27629 ID (9)	5	Test b done 10 mo after test a with aztreo reagents only
19c				MDM ID (8) Ticar ID 20 µg (8) Ceftaz ID 2 mg (8) SQ 27629 (8) Arginine ID (8)	5	Test c done 2 mo after test b
20	23/M	Azlo	61	Azlo prick 20 µg		
21	22/M	Pip	37	PPL ID (7.5) MDM ID (8) Pip ID 20 µg (8) Ceftaz ID 20 µg (8) Aztreo ID (7) SQ 27629 ID (8) SQ 26992 ID (8) Arginine ID (8)	4	Prior aztreo × two; ticar, azlo, tobra, negative

which was also used as negative control. Prick application of histamine phosphate, 0.275 mg/ml (0.1 mg/ml of histamine base, Eli Lilly & Co., Indianapolis, Ind.) was used as positive control. The ST reagent concentrations used ($\leq 10^{-2}$ mol/L) have been previously found not to cause nonspecific irritant reactions in normal nonallergic subjects.^{11, 20-24} In addition, four normal adult volunteers with no history of BLA allergy were tested with PPL, MDM, azlocillin, piperacillin, ticarcillin, ceftazidime, aztreonam, SQ 27629, SQ 26992, arginine, and controls at the above concentrations. In three cases, BLAs were also diluted in normal saline, and in two cases BLAs were additionally diluted in sterile distilled water; these were also applied without BLA as alternate diluents. Saline produced slightly larger (0 to 1.5 mm) wheals than PBS, whereas water produced much more pain, and in one case, distinctly larger (≥ 3 mm) wheals. In one case, arginine produced erythema without a positive wheal. No positive BLA reactions occurred.

In vitro assays

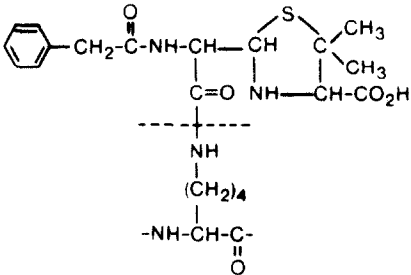
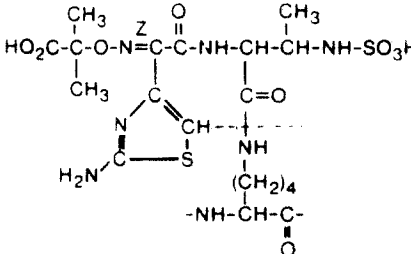
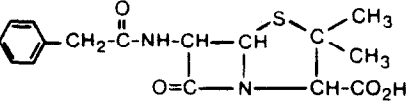
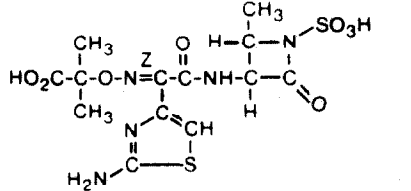
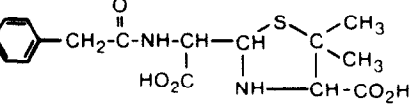
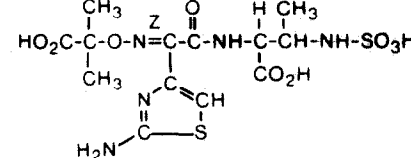
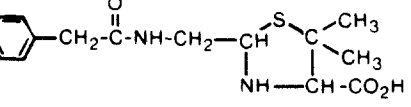
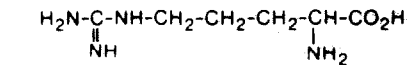
Blood samples for assay of aztreonam- and PPL-specific IgE and IgG antibodies were obtained at entry, on discharge from the hospital after completion of treatment, and at

follow-up 20 to 30 days after discharge. Serum was separated and stored in plastic tubes at $\leq 20^{\circ}$ C until testing. Serum IgG and IgE antibodies to PPL and aztreonam haptens conjugated to appropriate protein carriers were measured in the laboratory of Dr. N. Franklin Adkinson by previously described, double-antibody radioimmunoprecipitation and agarose-based RAST assays for IgG and IgE, respectively.^{10, 13} The specificity of positive RAST binding was investigated by RAST inhibition.¹⁸ The sensitivities of these assays are approximately 10 ng/ml for PPL-specific IgG, 150 pg/ml for PPL-specific IgE, 30 ng/ml for aztreonam-specific IgG, and 1 ng/ml for aztreonam-specific IgE.^{10, 12}

RESULTS

Study subjects

Twenty-one patients with CF and with a well-documented history of at least one immediate-onset allergic SR to an anti-PA BLA were studied. There were 11 male and 10 female patients, aged 5 to 39 years (mean $\pm$ SD, 22.5 ± 8.5 years). Patients were independently evaluated at three CF Centers (Louisiana State University at Shreveport: three male and two female patients, mean age, 16.8 ± 9.5 years;

 Benzylpenicilloyl-polylysine 6×10^{-5} M (penicilloyl)	 SQ 26,992-polylysine, or SQ 27,629 6×10^{-3} M (aztreonamyl)
 Benzylpenicillin* 10^{-2} M	 Aztreonam (with L-arginine buffer) 6×10^{-3} M (1.26×10^{-2} M)
 Benzylpenicilloste* 10^{-2} M	 SQ 26,992† 6×10^{-3} M
 Benzylpenicilloste* 10^{-2} M	 L-arginine++ 1.26×10^{-2} M

* Constituent of minor determinant mixture (MDM)

† Open-ring hydrolysis product of aztreonam

++ Solvent control

FIG. 1. Structures and concentrations of penicillin and analogous aztreonam-derived ST reagents. (Reprinted with permission from reference 11.)

range, 5 to 29 years; Children's Hospital of the King's Daughters, Norfolk, Va.: three male and two female patients, mean age, 23.2 ± 11.3 years; range, 14 to 36 years; Children's Hospital at Stanford; five male and six female patients, mean age, 24.8 ± 6.1 years; range, 16 to 40 years). There were no significant differences in sex or age between patients at the three centers. All patients had received multiple courses of anti-PA BLA before occurrence of an allergic reaction.

In each case, the reaction occurred during hospitalization for PA-associated exacerbation and was observed and documented in the chart. The BLA allergy histories and ST results are described in Table I. In all cases, the allergic reaction included cutaneous manifestations (pruritus, erythema, urticaria, and/or angioedema) occurring within 30 minutes after an intravenous dose of the primary drug. In 11 patients, additional manifestations included wheezing or

TABLE II. BLAs causing allergic reactions and nature of allergic reactions

Primary drug	
Ceftazidime	6
Piperacillin	5
Ticarcillin	6
Azlocillin	4
Other drugs	
Imipenem	2
Oxacillin	1
Carbenicillin	1
Timentin*	1
Cefoperazone	1
Reaction	
Cutaneous	21
Respiratory	8
Fever	2
Renal	1
Serum sickness	1

Reaction-evaluation time lapse: 32.5 ± 21.5 months (range, less than 1 week to 72 months).

*Beecham Laboratories, Bristol, Tenn.

stridor, fever, nephritis, and/or subsequent serum sickness.

There were four primary drugs: ceftazidime, piperacillin, ticarcillin, and azlocillin. In 12 patients, allergic reactions to other BLAs (oxacillin, imipenem, carbenicillin, ticarcillin-clavulanate (Timentin; Beecham Laboratories, Bristol, Tenn.), and cefoperazone) had previously occurred (Table II). At least four patients also had earlier histories of allergic reactions to oral penicillin; however, these reactions were not well documented.

Skin test results

Along with PPL and MDM, the primary drug was always used in the ST battery. In several cases, additional BLAs causing previous allergic reactions were included. Complete STs were positive for at least one BLA reagent in 19 of 21 patients (90%; Table III). The average time lapse between the primary drug reaction and skin testing was 32.5 ± 21.4 months, with a range of less than 1 week to 72 months. Two patients with positive histories but negative STs had time lapses between the primary drug reaction and skin testing of 43 and 65 months, respectively. Only one patient had a primary drug reaction within the month before skin testing; in this patient (Table I, No. 8), initially negative STs were positive when tests were repeated a month later.

The most common BLA reagent causing a positive ST was the primary drug, which was positive in 17

TABLE III. Summary of skin test results

ST responses	
19/21 patients	(90%) ST positive to PCN and/or cephalosporin reagent(s)
2 patients	ST negative (time lapse, 43 and 65 months)
Reagents causing positive ST	
Primary drug	17 (89% of ST positive patients)
	6 prick ST positive (ceftaz, 4; pip, 1; azlo, 1)
MDM	11 (58%)
	4 prick ST positive
PPL	6 (32%)
	1 prick ST positive
Aztreo	1 (5.3%) No previous aztreo exposure
	4 (20%) Previous aztreo exposure
No. of positive STs per patient	
Reagents	
1	8 (42% of ST positive patients)
	6 Primary drug (azlo, 3; ceftaz, 2; ticar, 1)
	1 MDM
	1 PPL
2	5 (26%)
3	4 (21%)
≥ 4	1 (5%)

ST, Skin test; *ceftaz*, ceftazidime; *pip*, piperacillin; *azlo*, azocillin; *aztreo*, aztreonam; *ticar*, ticarcillin.

(89%) of the ST-positive patients. In six of these patients, the primary drug was positive on prick test (Table III). MDM resulted in positive STs in 10 (53%) of the ST-positive patients; four of these patients were positive on prick testing. Six (32%) of the ST-positive patients reacted to PPL; one patient was positive to PPL on prick testing.

In eight of the ST-positive patients (42%), only one BLA reagent produced a positive ST response. In these eight patients, the primary drug was the reagent responsible for the ST response in six patients, MDM was the sole positive reagent in one patient, and PPL was the only positive reagent in the final patient. Thus, two ST-positive patients (11%) would have been missed by skin testing with the primary drug, and only one patient (5% of the ST-positive group) would have been missed by screening with PPL and primary drug.

When MWDs for BLA and control reagents were compared, two factors complicating ST interpretation were noted. First, intradermal negative control (PBS) MWDs averaged 3.8 ± 2.7 mm. The occurrence of negative control MWD ≥ 4 mm resulted in a need to broaden the primary criterion for interpretation of ST results. Therefore, when a negative intradermal control MWD ≥ 4 mm invalidated the a priori primary

criterion (see METHODS), a secondary criterion (MWD ≥ 3 mm larger than negative control) was used in categorizing results (Table I). Second, although positive intradermal BLA ($n = 41$) MWDs were, as expected, significantly larger than negative BLA ($n = 191$) MWDs (7.2 versus 4.2 mm, respectively; $p < 0.001$), the positive control (histamine) caused significantly larger MWDs (10.2 ± 1.5 mm) than positive BLA ($p < 0.001$). Thus, because BLA intradermal responses were often not impressive, some responses may have been missed by the above criteria.

Serum PPL-specific IgE and IgG antibodies

PPL-specific IgE antibody in vitro test results were negative in all patients' sera, including sera from six patients who were ST positive to PPL. Serum samples from four of six PPL ST-positive patients demonstrated no RAST binding above background (< 0.13 ng/ml); in one PPL ST-positive patient, low levels of RAST binding (≤ 0.33 ng/ml) were not inhibited by soluble PPL, suggesting cross-reactive antibody or nonspecific binding. (Blood was not available for PPL-specific antibody assays in the sixth PPL ST-positive patient.) Increased PPL RAST binding not inhibitable by soluble PPL was also noted in samples from three PPL ST-negative patients. PPL-specific IgG antibodies were found at levels of 10 to 83 ng/ml in most patients tested. Five patients, however, were negative for detectable serum PPL-specific IgG.

Serum aztreonam-specific IgE and IgG antibodies

As with PPL-specific IgE, serum aztreonam-specific IgE antibodies were not detectable in vitro. Samples from three study subjects after allergic reactions to aztreonam (see below) were also negative. In addition, no circulating aztreonam-specific IgG antibodies were detectable. Lack of exposure could not account for these results because all patients had samples obtained after aztreonam therapy included in the assays. Six patients received multiple courses of aztreonam before in vitro testing. Thus, by in vitro criteria, aztreonam appeared less immunogenic in vivo than other BLAs.

Aztreonam cross-reactivity to BLA

Eighteen of 19 BLA ST-positive patients were treated with intravenous aztreonam. In addition, the two history-positive but ST-negative patients were also treated with aztreonam on the basis of favorable microbial susceptibility. Thus, 20 of 21 subjects received intravenous aztreonam. As reported elsewhere in detail, aztreonam was free of serious side effects and resulted in significant clinical improvement.²⁵

One patient (Table I, case No. 17), a 20-year-old atopic woman, was not treated with aztreonam because of positive ST results to aztreonam reagents. Notably, she had never received aztreonam before the ST evaluation. Between 1980 and 1985, she was treated with carbenicillin once and ticarcillin twice without incident, as well as oral dicloxacillin. In 1985 she developed drug fever on day 11 of her first course of piperacillin. She continued to tolerate dicloxacillin. Later, in 1985, drug fever recurred when dicloxacillin was switched to oral cefaclor. She also developed urticaria and fever 1 day after intravenous desensitization to piperacillin.¹⁶ She was then desensitized to cefoperazone without incident. In 1986 she developed wheezing 2 days after her fourth desensitization to cefoperazone. Another attempt at piperacillin desensitization resulted in fever and palatal edema. Between late 1986 and early 1989, she received six courses of ceftazidime after desensitization, and azlocillin after desensitization one time without adverse reactions. When she was skin tested in March 1989, she had a positive prick test to piperacillin; ceftazidime was not tested (Table I, No. 17a). She also had a positive intradermal reaction to SQ 27629. STs to aztreonam reagents were repeated in June 1989 with intradermal reactivity to SQ 27629 confirmed (Table I, No. 17b).

Sensitization during first treatment with aztreonam

Aztreonam was initially tolerated in 19 of 20 subjects treated. However, one patient, a nonatopic 40-year-old woman, was clinically sensitized during her first treatment with aztreonam. In 1982, after previous treatment with ampicillin, cloxacillin, and carbenicillin, she developed generalized urticaria on day 10 of her first course of ticarcillin. Skin testing in 1983 demonstrated local positive intradermal reactions to 10^5 U/ml of PCN G and 200 mg/ml of ticarcillin, followed by an SR requiring treatment. She was then desensitized to ticarcillin, with pruritus during the procedure and intermittently for the next 9 days. Between 1983 and late 1988, she was desensitized to cefoperazone four times, and imipenem, piperacillin, and ceftazidime one time each without incident. Skin testing in October 1988 demonstrated a positive prick test to MDM and a positive intradermal test to ticarcillin (Table I, No. 14a). Although she initially tolerated aztreonam, bronchospasm occurred on day 9 of treatment and worsened despite increasing bronchodilator and steroid therapy during the next 3 days. Aztreonam was stopped with improvement in airflow within 24 hours. She was desensitized to ceftazidime without incident on day 21 and was discharged on day 38. She was subsequently treated with ceftazidime after

desensitization without reaction in early 1989. On repeat skin testing in August 1989, she was again positive to prick test with MDM. Positive intradermal tests responses again occurred with ticarcillin and also azlocillin. Intradermal testing with aztreonam was now also positive (Table I, No. 14b). In vitro tests on blood drawn before, during, and after aztreonam treatment failed to detect PPL-specific IgG or IgE, or aztreonam-specific IgG or IgE.

Allergic reactions on reexposure to aztreonam

Three of the remaining 19 patients who initially tolerated aztreonam were sensitized by this treatment.

A 29-year-old atopic woman with a history of penicillin allergy had received several uneventful courses of cefazolin and cefamandole between 1979 and 1983. In 1983, she was ST positive to 10^3 U/ml of intradermal PCN G. She was treated with piperacillin uneventfully after intravenous desensitization. She received piperacillin after desensitization without incident again in 1984, but on her next admission, she developed urticaria 3 days after desensitization. Similar symptoms occurred in 1985 on her next admission 2 days after piperacillin desensitization. She was desensitized to azlocillin successfully twice in 1986, but in 1987, she reacted to azlocillin after the last desensitizing dose with pruritus, conjunctival edema, urticaria, palatal itching, and bronchospasm. She was then desensitized to ceftazidime without a reaction. During 1987 and 1988, she received ceftazidime uneventfully after desensitization five times, but developed serum sickness 6 days after desensitization with imipenem. She was then treated with aztreonam with no reaction. STs in July 1988 were negative (Table I, No. 19a). She was treated with aztreonam again without adverse reactions. However, on her next admission in October 1988, she developed a pruritic rash during the first dose of aztreonam. The next day she was desensitized to aztreonam without incident. A week later, because of a change in bacterial susceptibility, aztreonam was stopped. She was desensitized to piperacillin, but shortly after receiving the last desensitizing dose of piperacillin, she had angioedema and bronchospasm. She was treated again with aztreonam after desensitization in February and April 1989 without adverse reactions. In May 1989, STs to aztreonam reagents resulted in a positive reaction to SQ 27629 (Table I, No. 19b). In vitro tests on blood drawn before, during, and after reacting to aztreonam were positive for PPL-specific IgG but negative for PPL-specific IgE, aztreonam-specific IgG, and aztreonam-specific IgE. Skin testing in August 1989 demonstrated positive reactions to intradermal MDM, ticar-

cillin, ceftazidime, SQ 27629, and arginine (Table I, No. 19c).

A 16-year-old atopic female adolescent was diagnosed with allergic bronchopulmonary aspergillosis in 1984. During 1985 to 1987, she was treated with ticarcillin four times, ceftazidime and imipenem twice each, and azlocillin one time. On her first exposure to imipenem, she developed a pruritic rash on day 13 of treatment. The next time she received imipenem, she developed urticaria and angioedema during the desensitization procedure. In 1988 she had angioedema to ceftazidime; the next day she had urticaria and angioedema during desensitization to ceftazidime. In August 1988 she was ST positive to ceftazidime; imipenem was not tested (Table I, case No. 11a). She was treated with aztreonam. On her next admission in October 1988, she developed angioedema and bronchospasm during infusion of the first dose of aztreonam. She then underwent intravenous desensitization to aztreonam without reactions and received an uneventful course. She was treated three times with aztreonam after desensitization without problems in early 1989. In May 1989 she was skin tested again to aztreonam reagents; at this time, she had a positive intradermal reaction to SQ 26992 (Table I, No. 11b). In vitro tests on blood drawn before receiving aztreonam, after receiving aztreonam but before the allergic reaction, and after the reaction were negative for PPL- and aztreonam-specific IgE or IgG.

A 22-year-old atopic man had a history of ill-defined and undocumented allergic reactions to penicillin and carbenicillin. During his first three admissions at Children's Hospital-Stanford (1980 to 1984), he received intravenous cephalothin, ticarcillin, piperacillin, and oral dicloxacillin without reactions. However, in 1985 he had bronchospasm to piperacillin. The following day he had bronchospasm during desensitization to cefoperazone. The next day, another attempt at desensitization with cefoperazone failed again. He was subsequently treated after desensitization with imipenem, ticarcillin, and aztreonam twice each without adverse reactions. STs in August 1989 (Table I, No. 21) demonstrated positive intradermal reactions to PPL, MDM, piperacillin, ceftazidime, aztreonam, SQ 27629, SQ 26992, and arginine. Tobramycin, ticarcillin, and azlocillin were negative.

DISCUSSION

The risk for an allergic reaction to a BLA is known to be affected by a number of variables, including the nature, intensity, and persistence of immunologic responses, end organ sensitivity ("constitutional component"), the presence of atopy, the age of the subject,

the pattern and route of drug exposure, structural similarity and subsequent immunologic cross-reactivity of different BLAs, and inherent differences in immunogenicity among BLAs.^{3, 4, 26} Unfortunately, the interactions and mechanisms whereby these many variables affect clinical allergic manifestations to BLA are poorly understood.

Previous studies have documented allergic sensitization and clinical reactions to various BLAs in patients with CF. Brown et al.²⁷ described a patient with CF allergic to ticarcillin who was ST positive to ticarcillin and PPL. We previously reported a series of 11 BLA-allergic patients with CF, most of whom reacted preferentially to the primary drug on skin testing.¹⁶ Hilman and Rao²⁸ discussed desensitization in a series of azlocillin-allergic patients with CF. Earl and Sullivan²⁹ reported on a patient with CF allergic to ticarcillin, cefaclor, and cefoperazone who was ST positive to PPL but not PCN G, ticarcillin, or cephalothin. In the present study, to assess the cross-reactivity of aztreonam with other antipseudomonal BLAs, STs with PPL, MDM, and primary drug were conducted on history-positive patients. Seventeen of 19 (89%) ST-positive BLA-allergic patients with CF reacted to the primary drug. Most patients (53%) were also positive to MDM, but only a third were positive to PPL. This distinctive pattern of ST reactivity most likely reflects the unique spectrum of anti-PA BLAs to which patients with CF are repetitively exposed.

Aztreonam, the first monocyclic BLA to be used clinically, has been found to be less immunogenic in animals than other BLAs; in addition, IgE antibodies to other BLAs do not bind aztreonam, and antibodies raised to aztreonam do not generally cross-react with antibodies to other BLAs.¹⁰⁻¹² Several previous studies have suggested that these encouraging indications of safety could extend to clinical treatment of BLA-allergic human subjects with aztreonam.¹³⁻¹⁵ Indeed, a general lack of cross-reactivity between aztreonam and other anti-PA BLAs was demonstrated in this study. Nineteen of 20 high-risk BLA-allergic patients with CF were ST negative to aztreonam and tolerated initial treatment with aztreonam without clinical reactivity. Moreover, serologic responses (aztreonam-specific IgG or IgE) could not be demonstrated. However, one of 19 BLA ST-positive patients (No. 17) demonstrated evidence of preexisting cross-reactivity in that STs to aztreonam metabolites were positive before any exposure to aztreonam. Repeat skin testing confirmed these responses. This finding appears to be the first instance in which such cross-reactivity has been demonstrated.^{11, 13} This finding implies that caution is necessary when BLA-allergic patients with CF (if not other human subjects) are being treated with aztreonam.

In addition, a substantial allergenicity of aztreonam in BLA-allergic patients with CF was clearly evident in this study. In one case (No. 14), clinical sensitization occurred during the first course of treatment with aztreonam. This patient also converted STs with aztreonam reagents from negative before aztreonam therapy to positive when STs were repeated 9 months later. Two additional BLA-allergic patients with CF (Nos. 11 and 19) had no clinical reactions during their first and second respective lifetime exposures to aztreonam, but then they had immediate-onset hypersensitivity SRs on subsequent exposure. Aztreonam ST responses were found on repeat testing after clinical reactions in both patients. A fourth BLA-allergic patient (No. 21) was ST positive to aztreonam reagents when he was tested 1 month after his second treatment course with aztreonam; in this case, no clinical reaction had occurred, perhaps because aztreonam had been administered via precautionary intravenous desensitization. Thus, in this study, 20% of high-risk BLA-allergic patients with CF treated with aztreonam were sensitized by treatment.

Despite the substantial risk of sensitization to aztreonam (as well as other BLAs) in patients with CF with a previous allergic reaction to a BLA, the present study does not establish BLA skin testing as a reliable means of predicting reactivity. One disappointment was that ST reactivity could not be corroborated *in vitro* by combined RAST/RAST-inhibition studies. It is possible that positive PPL and/or aztreonam STs with negative *in vitro* results could be due to the greater sensitivity of intradermal skin testing for detection of low levels of IgE antibodies. However, even penicillin ST-negative subjects with a history of an SR are at elevated risk for clinical reactions on reexposure, indicating that the "gold standard" in BLA allergy remains the clinical observation of reactivity on challenge, despite our desire for reliable ancillary *in vivo* or *in vitro* diagnostic procedures.³ Another possible basis for discrepancies between skin and *in vitro* test results could arise from nonimmunologic constitutional factors, such as end organ reactivity or mast cell releasability.

Perhaps the most difficult issue raised by these data is the determination of optimal criteria for interpreting intradermal ST results. In several subjects, the unimpressive size of some BLA wheals coupled with larger than expected negative control (PBS) wheals suggests that distinguishing positive from negative intradermal STs is not always straightforward, a difficulty that has been remarked on previously in editorial discussion of BLA ST interpretation.³⁰ A number of varying criteria for distinguishing positive from negative BLA ST results have been offered,^{4, 17, 18, 20-22, 24} and several of these criteria were

assessed before deciding on the criteria described in METHODS to report the experimental results. The fact that patients 17 and 21 had positive ST reactions to aztreonam reagents without a history of clinical reactivity can be explained in the first case by presumptive cross-reactivity, and in the second case by desensitization before therapy with aztreonam that the patient received before the ST assessment; but these explanations are speculative. More troubling are the positive ST responses to arginine (the aztreonam solvent solution) in patients 14, 19, and 21. In one case (No. 14), intradermal arginine and PBS wheals were each just on opposite sides of the primary cutoff value of 4 mm. In the other two cases, intradermal arginine wheals were roughly as large as aztreonam wheals and ≥ 3 mm larger than PBS responses. These results raise the possibility of not only true sensitization to a nontherapeutic component of the drug vehicle (certainly not unprecedented in drug allergy) but also the possibility that more stringent criteria for a positive reaction may need to be used. The former appears more likely in view of the fact that no arginine reactions occurred in the absence of reactions to aztreonam or aztreonam metabolite. However, to resolve the issue of ST reliability, we plan additional studies of BLA reactivity in patients with CF that include a quantitative (planimetric) approach to ST measurement and assessment of in vitro cellular responses to BLA antigens, as well as circulating IgE antibodies. In addition, study of nonallergic patients with CF and investigation of in vivo and in vitro cellular responses to nonimmunologic stimuli are needed to understand more fully the important clinical phenomena of multiple BLA drug sensitivity in CF.

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Neuropeptide Y, a putative cotransmitter in noradrenergic neurons, induces mast cell degranulation but not prostaglandin D_2 release

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*Recent evidence suggests that neural transmitters, including neuropeptides, may modulate the release of mast cell mediators. Because neuropeptide Y (NPY) has recently been recognized as a putative cotransmitter in noradrenergic neurons, we studied the effect of NPY on purified rat peritoneal mast cells. NPY induced mast cell degranulation, as assessed by a dose-dependent increase in net release of β -hexosaminidase. The concentration that produced 50% of the maximal effect, approximately 10 $\mu\text{mol/L}$, evoked a 40% $\pm$ 3% release. As previously reported for other neuropeptides, release was fast with maximal release already achieved at 60 seconds. Release was at 4° C. In contrast to its effects on mast cell degranulation, NPY had no effect on the generation of prostaglandin D_2 , the major mast cell cyclooxygenase product. By comparison, the calcium ionophore A23187, at doses (4 $\mu\text{mol/L}$) that evoked comparable release of β -hexosaminidase, stimulated a net release of 37 $\pm$ 9 ng of PGD_2 per 10⁶ mast cells. These results raise the possibility that NPY may act as a modulator between the autonomic nervous system and mast cells. The results also imply that with neuropeptide stimulation, the release of preformed and newly formed mast cell mediators are mediated through independent pathways. (*J ALLERGY CLIN IMMUNOL* 1991;87:88-93.)*

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An interrelation between mast cells and nerve structures has long been recognized. Histologically, "mucosal" mast cells are in close proximity to nerve endings and blood vessels.¹ Nerve stimulation has been demonstrated to cause degranulation of mast cells in the skin² that contain mostly "serosal" mast cells.³ Nerve stimulation also results in the release of histamine from guinea pig atrium and rat ileum, in which there is no evidence for histaminergic neurones.⁴ It has been postulated therefore that mast cells are involved in neurogenic inflammation.

There are also pathologic conditions characterized by mast cell activation in the absence of obvious al-