Original Article

Prospective Piperacillin Lymphocyte Transformation Testing in Patients With Cystic Fibrosis Receiving Regular and Desensitization Courses of Piperacillin-Tazobactam

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What is already known about this topic? Piperacillin-tazobactam exposure to patients with cystic fibrosis is associated with a high frequency of T cell–mediated hypersensitivity reactions.

What does this article add to our knowledge? The lymphocyte transformation test (LTT) is a sensitive and specific assay for the diagnosis of piperacillin hypersensitivity in patients with cystic fibrosis. Desensitization results in attenuation of the drug-specific T-cell response.

How does this study impact current management guidelines? In our hospital we currently use the LTT to confirm a diagnosis of piperacillin hypersensitivity. In the future it might be feasible to use the LTT in tolerant patients before elective drug use and in allergic patients before drug (re)challenge or desensitization.

BACKGROUND: Piperacillin-tazobactam is used in patients with cystic fibrosis to treat recurrent respiratory infections. Exposure is associated with a high frequency of nonimmediate hypersensitivity.
OBJECTIVE: To assess the applicability of the lymphocyte transformation test (LTT) for the diagnosis of piperacillin hypersensitivity and the influence of desensitization on piperacillin-specific T-cell responses.
METHODS: Study arm 1 was an analysis of LTT responses from 58 naive/baseline tolerant patients with samples collected over a 3-year interventional phase. In study arm 2, 17 hypersensitive patients were recruited and LTTs were conducted before and after desensitization. Clinical hypersensitivity reactions in both arms were monitored over an 8-year observational period.
RESULTS: In study arm 1, 58 patients received 611 piperacillin-tazobactam courses (range, 2-40; mean ± SD, 10.5 ± 8.1) during the interventional phase; 11 patients developed hypersensitivity. The patients who remained tolerant received 236 piperacillin-tazobactam courses in the observational period, 9 of whom developed hypersensitivity. Ten of 11 interventional phase hypersensitive patients had a positive LTT whereas one remained negative. We recorded 136 negative LTTs with 39 tolerant patients, whereas eight patients had a positive LTT and four developed hypersensitivity during the observational period. Ten LTT-positive patients in study arm 2 underwent piperacillin-tazobactam desensitization, with seven tolerating the drug. The strength of the LTT decreased during desensitization, and negative results were recorded for a minimum of 14 days. During follow-up, eight patients tolerated 62 piperacillin-tazobactam courses through desensitization.
CONCLUSIONS: The LTT is a sensitive marker of drug sensitization that could be used to inform future patient management. Desensitization is associated with attenuation of the piperacillin-specific T-cell response. © 2024 The Authors.

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Abbreviations used
CFSE- carboxyfluorescein succinimidyl ester
LTT- Lymphocyte transformation test
PBMC- Peripheral blood mononuclear cell
SI- Stimulation index

Key words: *Human; T cells; Drug hypersensitivity; Desensitization*

INTRODUCTION

Cystic fibrosis is an autosomal recessive disease caused by mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene that codes for an anion channel that regulates chloride and bicarbonate ion transport across epithelial cell membranes. An absence or dysfunction in the CFTR protein results in dehydrated airway surface liquid, thickened secretions, and defective mucociliary clearance. As a result, patients develop recurrent respiratory infections driving progressive lung damage and premature death.[1,2] β-Lactam antibiotics are frequently used to treat recurrent gram-negative bacterial infections such as *Pseudomonas aeruginosa*. Although the drugs are highly effective, their use can be hindered by a high incidence of antibiotic hypersensitivity,[3-6] which in the setting of cystic fibrosis is likely attributable to cumulative drug exposure, accentuated inflammation, and the presence of a hyperimmune state.

Most β-lactam antibiotic–induced nonimmediate adverse reactions present as rashes, arthralgia, and fever.[3-7] Identification of drug-responsive T cells in both the peripheral circulation and immune-targeted organs of patients implicates these effector cells in the reaction pathogenesis.[8-13] Piperacillin-tazobactam, a β-lactam–β-lactamase combination, is commonly used in patients with cystic fibrosis. Patients receive 12 g/1.5 g daily infusions for 2 weeks. Before the introduction of CFTR modulator triple therapy, it was common for patients to receive multiple piperacillin-tazobactam courses per year. Piperacillin-tazobactam use in patients with cystic fibrosis is associated with the onset of hypersensitivity reactions in up to 50% of those who receive it, compared with fewer than 10% in the general population.[3,5,6,14] Most reactions are attributable to the piperacillin component of the drug combination.[15,16] We previously characterized T cells from piperacillin-hypersensitive patients that were responsive to piperacillin-albumin conjugates and activated via an MHC- and processing-dependent mechanism.[9]

In the multi-hypersensitive patient group in whom alternative treatment strategies have been exhausted, desensitization represents an effective method of reintroducing the drug. The procedure involves gradually increasing a suboptimal dose until a therapeutic dose is achieved and, most important, tolerated.[7] Little is known regarding the immunologic basis of a successful desensitization in nonimmediate hypersensitivity reactions. It is possible that desensitization successes might be seen only in patients who incorrectly receive the diagnosis of being hypersensitive.

The lymphocyte transformation test (LTT) is a simple assay that quantifies expansion of memory drug-specific T cells in hypersensitive patients.[17-21] Our previous retrospective study of 28 patients with cystic fibrosis and nonimmediate hypersensitivity who were exposed to piperacillin-tazobactam revealed an LTT sensitivity of 76% (positive LTT in hypersensitive cohort), with only four individuals displaying positive skin test results.[22] Piperacillin-specific T-cell responses were not detected in 10 tolerant controls; thus, the specificity for the assay (negative responses in tolerant cohort) was 100%. The LTT sensitivity in other patient groups varied from 58% to 89% depending on the drug class, clinical phenotype of the adverse event, and time of blood sampling.[17-21] Correspondingly, assay specificity ranged from 93% to 100%.[17-21] As reported,[23] it is possible that negative LTT results in hypersensitive patients are related to an incorrect clinical diagnosis, although confirmatory challenge testing is rarely performed.

The objectives of this study were to monitor LTT responses prospectively in (1) baseline tolerant patients (on recruitment) during piperacillin-tazobactam therapy over a three-interventional phase with an 8-year observational phase, and (2) hypersensitive patients re-exposed to piperacillin-tazobactam via desensitization to assess whether a graded increase in drug exposure modulates the drug-specific T-cell response. A better understanding of both the performance of the LTT for hypersensitivity diagnosis and the immunologic mechanisms employed for successful desensitization will assist future patient management and reduce patient morbidities associated with drug exposure.

METHODS

Patient sampling and desensitization protocol

All patients registered at the regional adult cystic fibrosis unit in Leeds, irrespective of allergy status, were invited to participate in the study. Eighty-five patients were enrolled in the study with informed written consent under approval from the Leeds research ethics committee (Prospective Study Investigating Hypersensitivity Reactions to Beta-lactam Antibiotics; IRAS ID: 97797; UKCRN ID: 13111). A total of 58 piperacillin-tazobactam baseline tolerant patients were included in study arm 1, 17 piperacillin-tazobactam hypersensitive patients were included in study arm 2, and 10 were excluded from the study because blood samples were not taken for LTT analysis. Clinical staff and patients were blinded to LTT testing results during the interventional and observational phases of the study.

For study arm 1, 57 of 58 patients reported a history of one or more piperacillin-tazobactam treatment courses. Patient demographic details, allergy history, prestudy piperacillin/tazobactam history, and observational phase piperacillin-tazobactam treatment courses are described for tolerant and hypersensitive patients in Table E1 (in this article's Online Repository at www.jaci-inpractice.org) and Table I, respectively. Table II lists observational phase outcomes of study arm 1 tolerant patients. Patients donated 20- to 40-mL blood samples on recruitment; repeated sampling was then conducted over a 3-year interventional phase before and immediately after 14-day treatment with piperacillin-tazobactam. Additional samples were taken (1) immediately, on diagnosis of an acute hypersensitivity reaction; and, when available, (2) 1 to 3 months after the resolution of clinical symptoms. Sample collection (interventional phase) took place in April 2012 to March 2015, with

TABLE I. Characteristics of hypersensitive patients in study arm 1

Patient*	Age/sex	FEV_1 (%)	Body mass index	Allergies at baseline and during sample collection	Study arm	LTT result
008	31/M	46	18.4	Aztreonam (2008) maculopapular rash (after eight courses) Piperacillin-tazobactam (2012) rash (after five courses)	Prospective	Positive acute LTT
009	18/F	69	19.5	Ceftazidime (2011) rash (after 15 courses) Piperacillin-tazobactam (2013) drug fever and unwell (after four courses)	Prospective	Positive acute and post-reaction LTT
013	22/F	51	17.4	Flucloxacillin (2007) maculopapular rash (after three courses) Piperacillin-tazobactam (2012) rash (after 21 courses)	Prospective	Positive acute LTT
032	30/M	85	23	Meropenem (2008) delayed urticarial reaction (after 16 courses) Piperacillin-tazobactam (2013) maculopapular rash (after three courses)	Prospective	Positive post-reaction LTT
033	34/M	56	22.6	Meropenem (2007) delayed urticaria reaction (after eight courses) Piperacillin-tazobactam (2015) maculopapular rash and unwell (after 12 courses)	Prospective	Positive acute LTT
034	31/F	35	18.2	Meropenem (2012) urticaria (after two courses) Piperacillin-tazobactam (2015) fever and maculopapular rash (after four courses)	Prospective	Positive post-reaction LTT
061	28/M	58	27.5	Aztreonam (2011) urticaria (after six courses) Piperacillin-tazobactam (2014) drug fever and unwell (after six courses)	Prospective	Positive acute LTT
073	30/F	56	23.4	Aztreonam (2014) urticarial reaction (after five courses) Piperacillin-tazobactam (2014) drug fever and arthralgia (after three courses)	Prospective	Positive acute LTT
077	30/M	92	21.7	Piperacillin-tazobactam (2014) drug fever and arthralgia (after seven courses)	Prospective	Positive acute and post-reaction LTT
083	27/F	59	19.7	Amoxicillin (2008) maculopapular rash (after 12 courses) Piperacillin-tazobactam (2015) maculopapular rash and fever (after three courses)	Prospective	Positive acute LTT
015	19/M			Piperacillin-tazobactam (2013) mild lip swelling on day 1 (after two courses)	Prospective	Negative acute and post-reaction LTT

LTT, lymphocyte transformation test.
*Patients 7, 36, 51, 53, 59, 67, 69, 78, 79, and 84 were excluded from study (and tables) because samples for LTT were not obtained.

TABLE II. Observational phase outcomes of interventional phase tolerant patients

Observational phase outcomes	Interventional phase tolerant patients		
	Negative LTT	Positive LTT for patients exposed to treatment during interventional phase	Positive LTT for patients not exposed to treatment during interventional phase
Patients, n	39	5	3
Observational phase treatment courses	184	43	11
Observational phase adverse events	5 (13%)	1 (20%)	3 (100%)

LTT, lymphocyte transformation test.

piperacillin-tazobactam use and allergy status monitored until June 2023 (observational phase).

We recruited 17 patients with a history of piperacillin-tazobactam hypersensitivity to study arm 2. Table III lists basic demographics of patients and details of adverse reactions. Each patient donated a blood sample on recruitment to the study. Twelve of these patients were subjected to piperacillin-tazobactam desensitization in which a rapid intravenous seven step desensitization was performed. Each step was a 10-fold increase in concentration, with the final step being a therapeutic piperacillin (4 g piperacillin, 3 times/d)-tazobactam dose. The procedure lasted 2 hours 30 min. Blood was sampled at various time points (dependent on patient availability) during desensitization (1-3 h), during piperacillin-tazobactam treatment (1-14 days), and when possible afterward, to determine the effect of the procedure on the piperacillin-specific peripheral blood mononuclear cell (PBMC) proliferative response.

Figure 1, *A* is a flow diagram presenting an overview of the study design and summary of LTT results.

An material transfer agreement was in place for the transfer of fresh blood samples to Liverpool at room temperature. Peripheral blood mononuclear cells were isolated from each sample within 5 hours of venipuncture and LTTs were conducted immediately.

Peripheral blood mononuclear cell isolation and LTT

Peripheral blood mononuclear cells were extracted from peripheral blood by layering atop Lymphoprep (Axis-Shield, Dundee, Scotland). After centrifugation to enable density gradient separation, the PBMC layer was removed using a Pasteur pipette. During the procedure PBMCs were washed three times to ensure serum containing piperacillin-tazobactam was not present in the LTT. Peripheral blood mononuclear cells (1.5×10^5/well) were cultured in triplicate with titrated concentrations of piperacillin (0.125-4 mM) in a 96-well *U*-bottomed plate. On day 5, [^{3}H]thymidine (0.5 μCi/well) was added and plates were incubated for a further 16 hours before analysis of incorporated radioactivity using a MicroBeta TriLux 1450 LSC beta counter (PerkinElmer, Cambridge, UK).

Although a cutoff stimulation index (SI) of 2 or more or 3 or more for a positive LTT is often used, this value is not fully validated, and laboratory-specific cutoffs are sometimes employed. To exclude false-positive responses in tolerant and allergic patients we have applied a more vigorous system in which an SI of 2 or more was deemed positive only when the positive reading was observed at three consecutive drug concentrations. A positive LTT was then applied, in which + indicates an SI of 2 or greater or less than 3; ++, 3 or greater or less than 5; +++, 5 or greater or less than 10; and ++++, 10 or greater.

RESULTS

Study arm 1

The 58 patients recruited to study arm 1 received 611 (range, 2-40; mean ± SD, 10.5 ± 8.1) piperacillin-tazobactam courses on completion of the 8-year observational phase. This number covers piperacillin-tazobactam courses before the study, during the interventional phase, and during the observational phase follow-up. The recruitment cohort included two naive and 56 baseline tolerant patients, with a history of 0 to 32 previous piperacillin-tazobactam treatment courses. Patients were exposed to one to seven piperacillin-tazobactam courses during the interventional phase and none to 21 courses during the observational phase (Tables E1, I, and II). Twenty piperacillin-tazobactam hypersensitivity reactions were reported by the end of the study (11 interventional phase and nine observational phase). The average number of courses taken before the onset of hypersensitivity was 7.7 ± 5.0 (Figure 1, *B*). A total of 163 LTTs were conducted before or after treatment and when patients developed an adverse reaction (details discussed subsequently).

Patients who developed piperacillin hypersensitivity during the interventional phase

Eleven previously tolerant patients developed hypersensitivity during the interventional phase with the diagnosis made on an assessment of the clinical history (Table I). Ten reactions were classified as possible type IV nonimmediate reactions. One patient (P015) developed mild facial swelling 1 day after piperacillin-tazobactam exposure. Skin testing was not performed owing to the low sensitivity observed in our center.[7,22] Of 11 LTTs conducted when blood was sampled within 48 hours of diagnosis, eight were positive (two grade +/++, five grade +++, and one grade ++++). Piperacillin-specific PBMC proliferative responses (one grade ++ and one grade ++++) were detected in two of the three patients with negative acute sampling LTT when blood was sampled 1 or more months after symptoms subsided. Proliferation with piperacillin was dose-dependent; the strongest responses were detected at concentrations between 0.5 and 4 mM (Figure 2, *B* shows representative data from various time points for four hypersensitive patients from study arm 1 and two from study arm 2). Figure E1 (in this article's Online Repository at www.jaci-inpractice.org) shows time-dependent LTT assessments of two patients before, during, and after the hypersensitivity diagnosis.

Specific exceptional case descriptions

Patient P032 recorded a positive LTT on recruitment to the study despite a tolerant diagnosis at this point. The patient

TABLE III. Characteristics of allergic patients in study arm 2 and outcome of desensitization

Patient*	Age/sex	FEV_1 (%)	Body mass index	Allergies at baseline and during sample collection	Study arm	LTT result	Desensitization comments and history during observational period
001	32/F	42	23.5	Piperacillin-tazobactam (2008) drug fever and joint pains Ceftazidime (2003) maculopapular rash	Retrospective pre-desensitization	Positive acute and retrospective LTT	Tolerated one piperacillin-tazobactam course with desensitization but reacted on second course with fever and arthralgia on day 3. Samples not collected during desensitization. No subsequent piperacillin-tazobactam exposure
011	31/M	51	36	Piperacillin-tazobactam (2008) maculopapular rash, failed desensitization Meropenem (2013) maculopapular rash, failed desensitization	Retrospective pre-desensitization	Positive LTT	No subsequent piperacillin-tazobactam exposure
014	32/F	30	25.8	Piperacillin-tazobactam (2009) joint pains and fever	Retrospective pre-desensitization	Positive LTT	No subsequent piperacillin-tazobactam exposure
019	23/F	34	17.2	Piperacillin-tazobactam (2006) maculopapular rash and facial swelling	Retrospective pre-desensitization	Positive LTT	Tolerated 21 piperacillin-tazobactam courses with desensitization
020	27/M	17	18.7	Piperacillin-tazobactam (2008) drug fever on day 5	Retrospective pre-desensitization	Positive LTT	No subsequent piperacillin-tazobactam exposure

024	22/M	95	23.4	Piperacillin-tazobactam (2002) urticarial rash and drug fever Aztreonam (2003) maculopapular rash Meropenem (2008) maculopapular rash sulfamethoxazole-trimethoprim (2008) maculopapular rash	Retrospective pre-desensitization	Positive LTT	Failed desensitization with drug fever and arthralgia on day 5
043	42/M	19	21.5	Piperacillin-tazobactam (2008) maculopapular rash	Retrospective pre-desensitization	Positive LTT	Tolerated five piperacillin-tazobactam courses with desensitization
044	37/M	42	21.4	Piperacillin-tazobactam (2008) maculopapular rash and drug fever	Retrospective pre-desensitization	Positive LTT	Failed desensitization on day 2 with fever, maculopapular rash, and vomiting
049	28/F	46	19.6	Piperacillin-tazobactam (2013) maculopapular rash Meropenem (2011) fever and arthralgia Ceftazidime (2008) fever and arthralgia	Retrospective pre-desensitization	Positive LTT	Tolerated four piperacillin-tazobactam courses with desensitization
050	35/M	30	19.1	Piperacillin-tazobactam (2008) maculopapular rash Aztreonam (2005) fixed drug reaction Ceftazidime (2008) urticaria	Retrospective pre-desensitization	Positive LTT	Tolerated 18 piperacillin-tazobactam courses with desensitization
055	23/M	41	17.2	Piperacillin-tazobactam (2007) maculopapular rash Ceftazidime (2008) urticaria Meropenem (2011) urticaria	Retrospective pre-desensitization	Positive LTT	Tolerated one piperacillin-tazobactam course with desensitization
056	18/F	69	22	Piperacillin-tazobactam (2012) maculopapular rash and arthralgia	Retrospective pre-desensitization	Positive LTT	Tolerated six piperacillin-tazobactam courses with desensitization

(continued)

TABLE III. *(Continued)*

Patient*	Age/sex	FEV_1 (%)	Body mass index	Allergies at baseline and during sample collection	Study arm	LTT result	Desensitization comments and history during observational period
057	18/F	54	20.7	Piperacillin-tazobactam (2009) maculopapular rash	Retrospective pre-desensitization	Positive LTT	Tolerated one piperacillin-tazobactam course with desensitization
066	23/M	43	20.6	Piperacillin-tazobactam (2010) maculopapular rash Ceftazidime (2008) urticaria Temocillin (2016) drug fever and maculopapular rash	Retrospective pre-desensitization	Positive LTT	Tolerated four piperacillin-tazobactam courses with desensitization. Reacted during fifth desensitization on day 2 with maculopapular rash
081	23/F	36	19.9	Colomycin (2013) urticaria Piperacillin-tazobactam (2010) maculopapular rash, joint pains, and fever Meropenem (2011) maculopapular rash	Retrospective pre-desensitization	Positive LTT	Tolerated six piperacillin-tazobactam courses with desensitization
058	26/F	32	20.3	Piperacillin-tazobactam (2011) drug fever and arthralgia Ceftazidime (2007) urticaria Cotrimoxazole (2011) maculopapular rash	Retrospective pre-desensitization	Negative acute and retrospective LTT	Tolerated five piperacillin-tazobactam courses with desensitization
064	18/M	48	20.4	Piperacillin-tazobactam (2010) maculopapular rash Cotrimoxazole (2011) maculopapular rash	Retrospective pre-desensitization	Negative LTT	Tolerated two piperacillin-tazobactam courses with desensitization

LTT, lymphocyte transformation test.
*Patients 7, 36, 51, 53, 59, 67, 69, 78, 79, and 84 were excluded from the study (and tables) because samples for LTT were not recruited.

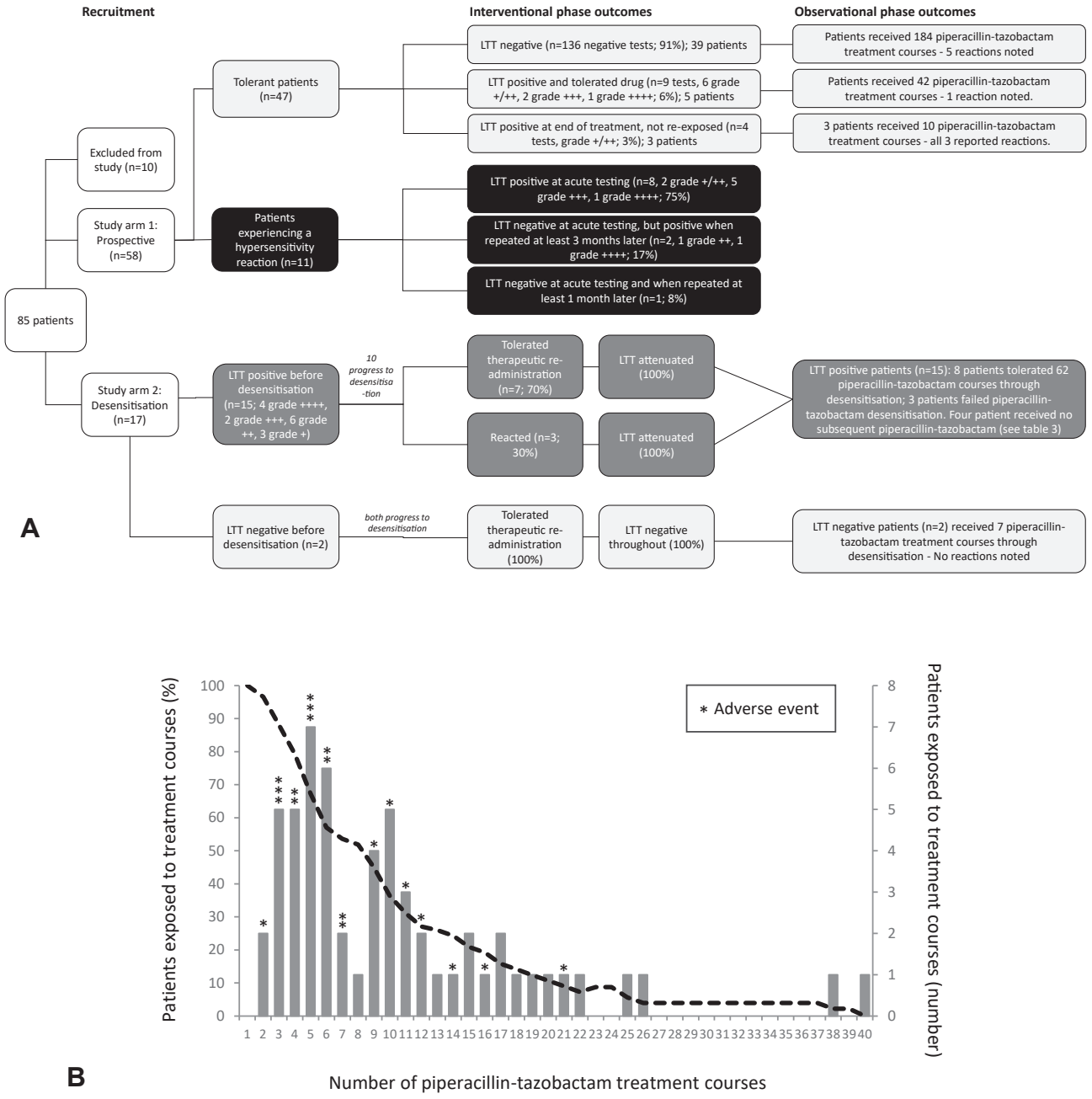


FIGURE 1. Study design, patient outcomes and lymphocyte transformation test (LTT) results. (**A**) Flow diagram showing study design; results of LTT testing in tolerant, hypersensitive, and desensitized patients; and observational phase study follow-up (lines connecting boxes link information relating to the same patients). Patients developing allergic reactions did not receive additional courses of piperacillin-tazobactam; thus, there are no data during the observational stage. (**B**) Number of piperacillin-tazobactam treatment courses received by study participants. *Asterisks* indicate timing of hypersensitivity reactions.

(P032) developed symptoms of hypersensitivity within 24 hours of the first subsequent piperacillin-tazobactam treatment course and the piperacillin LTT was negative at this time point. However, the LTT was positive 7 weeks later and the strength of the piperacillin-specific proliferative response was similar to that observed before the adverse event. Despite repeated testing, PBMCs from one hypersensitive patient (P015) were not activated with piperacillin. The patient developed facial swelling 1 day after piperacillin-tazobactam exposure with other signs of piperacillin hypersensitivity not present.

PATIENTS WHO SAFELY TOLERATED PIPERACILLIN-TAZOBACTAM TREATMENT

A total of 47 patients were classified clinically as piperacillin-tazobactam tolerant throughout the study. Moreover, 136 negative

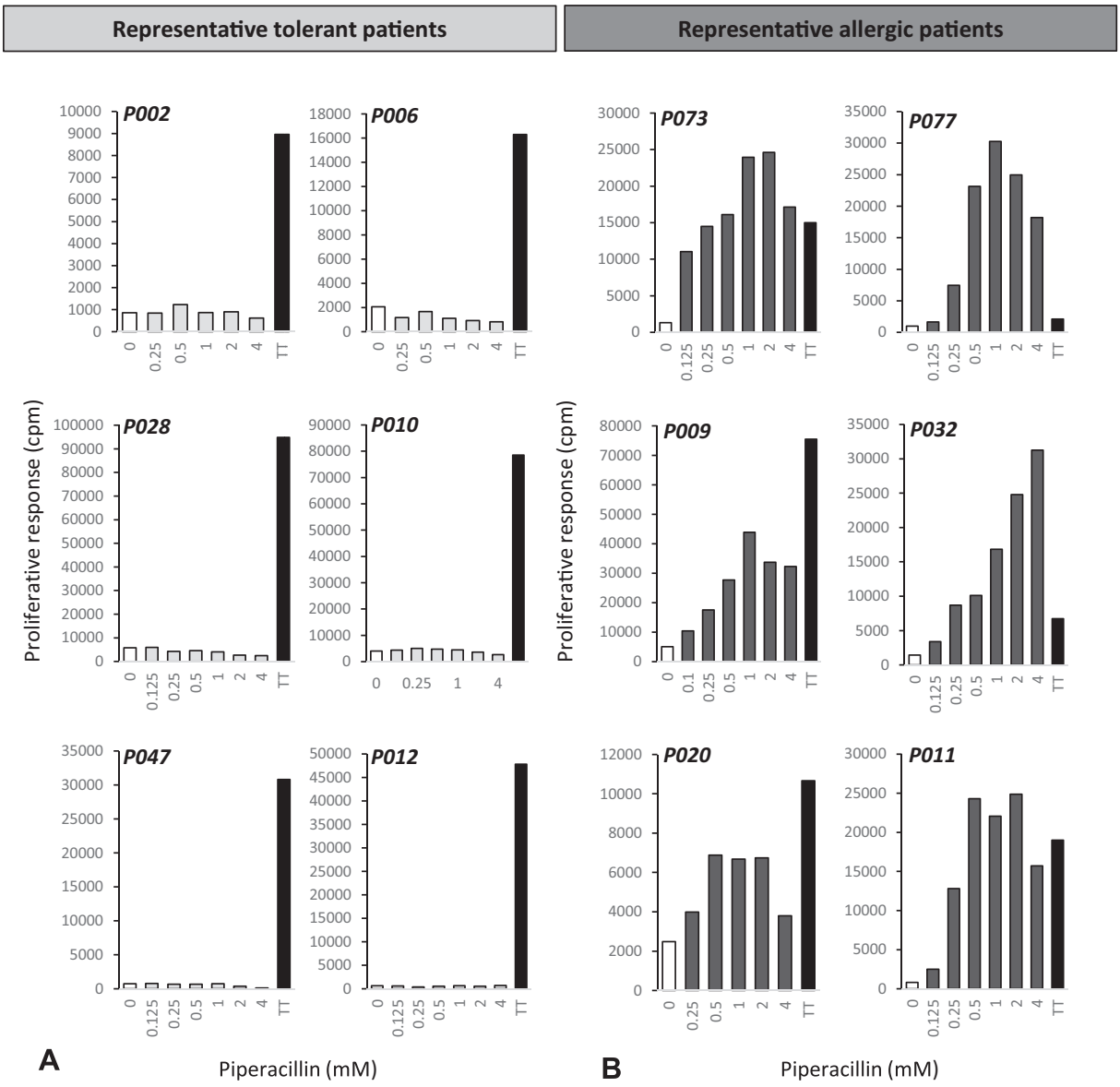


FIGURE 2. Piperacillin-induced stimulation of tolerant and hypersensitive peripheral blood mononuclear cell proliferation. Peripheral blood mononuclear cells from (**A**) tolerant and (**B**) hypersensitive individuals were incubated with titrated concentrations of piperacillin for 5 days in a 96-well *U*-bottomed plate (37°C; 5% CO_2). R9 medium and tetanus toxoid served as negative and positive controls, respectively. [^{3}H]Thymidine was added for 16 hours and proliferation was assessed by scintillation counting. Six representative tolerant and hypersensitive patients are shown.

LTTs were recorded from 39 tolerant patients. Figure 2, *A* shows representative negative LTT results from six patients in which the SI did not exceed 2 at any tested piperacillin concentration. Figure E1, B shows time-dependent LTT results from two tolerant patients receiving four to six piperacillin-tazobactam courses during the study. The PBMCs from patient P016 were tested on 12 separate occasions; 11 LTTs were negative whereas one posttreatment LTT was recorded as weakly positive at one piperacillin concentration. Because (1) there was an absence of a dose-dependent proliferative response, and (2) the patient safely tolerated several additional piperacillin-tazobactam treatment courses, the single-concentration LTT SI above 3 was likely false positive. After the interventional phase these patients received 184 regular piperacillin-tazobactam treatment courses over the 8-year observational phase, with only five hypersensitivity reactions reported (Tables E1 and II, and Figure 1).

Description of positive LTTs in tolerant patients

In total, nine positive piperacillin LTT responses were recorded in six patients who went on to tolerate a regular piperacillin-tazobactam treatment course safely. In four of these patients the positive LTT was weak graded as + or ++. In two patients, +++ or ++++ positive LTT responses were recorded before the patients tolerated piperacillin-tazobactam treatment. Figure 3, *A* shows LTT data from two of these patients.

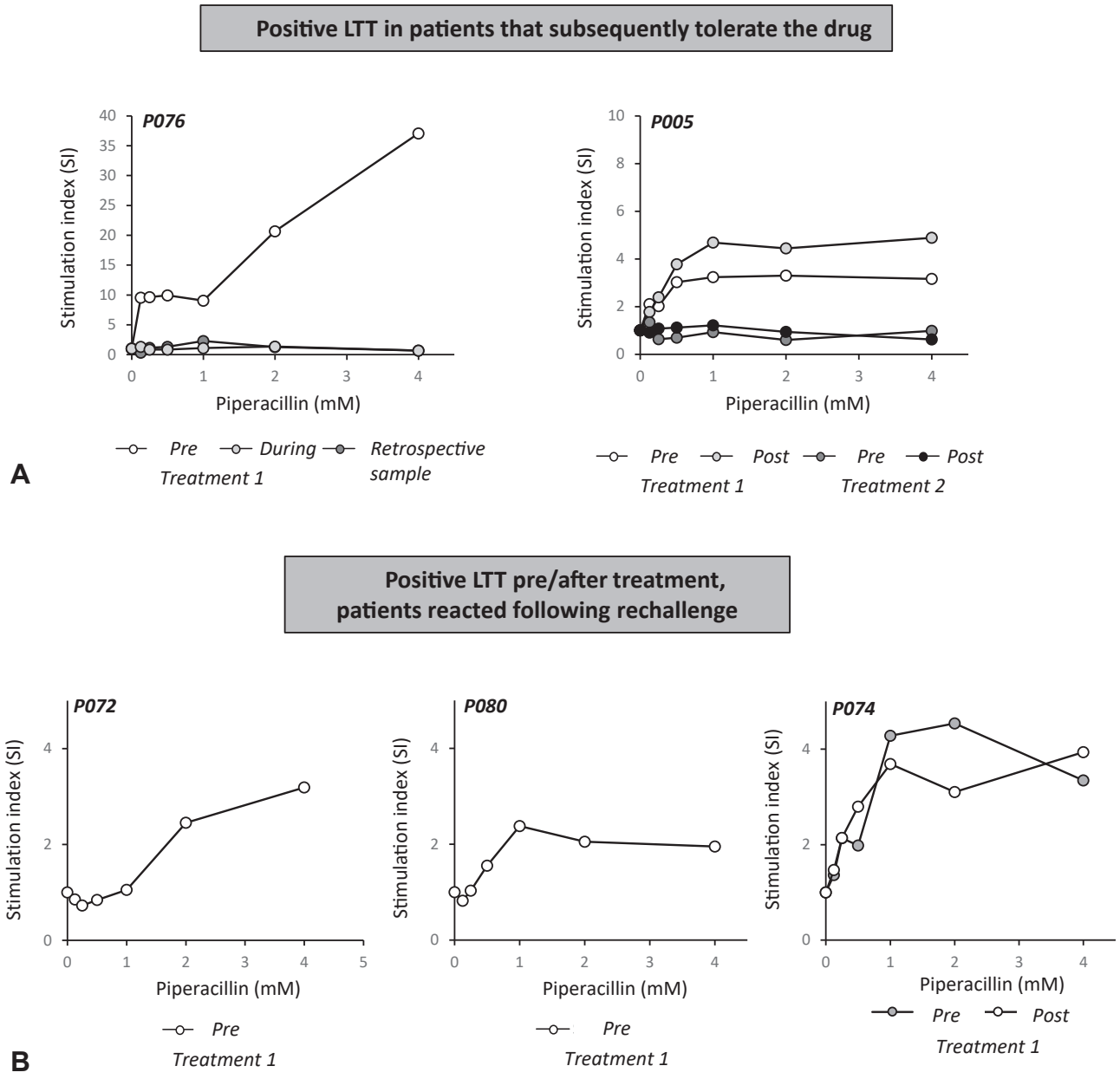
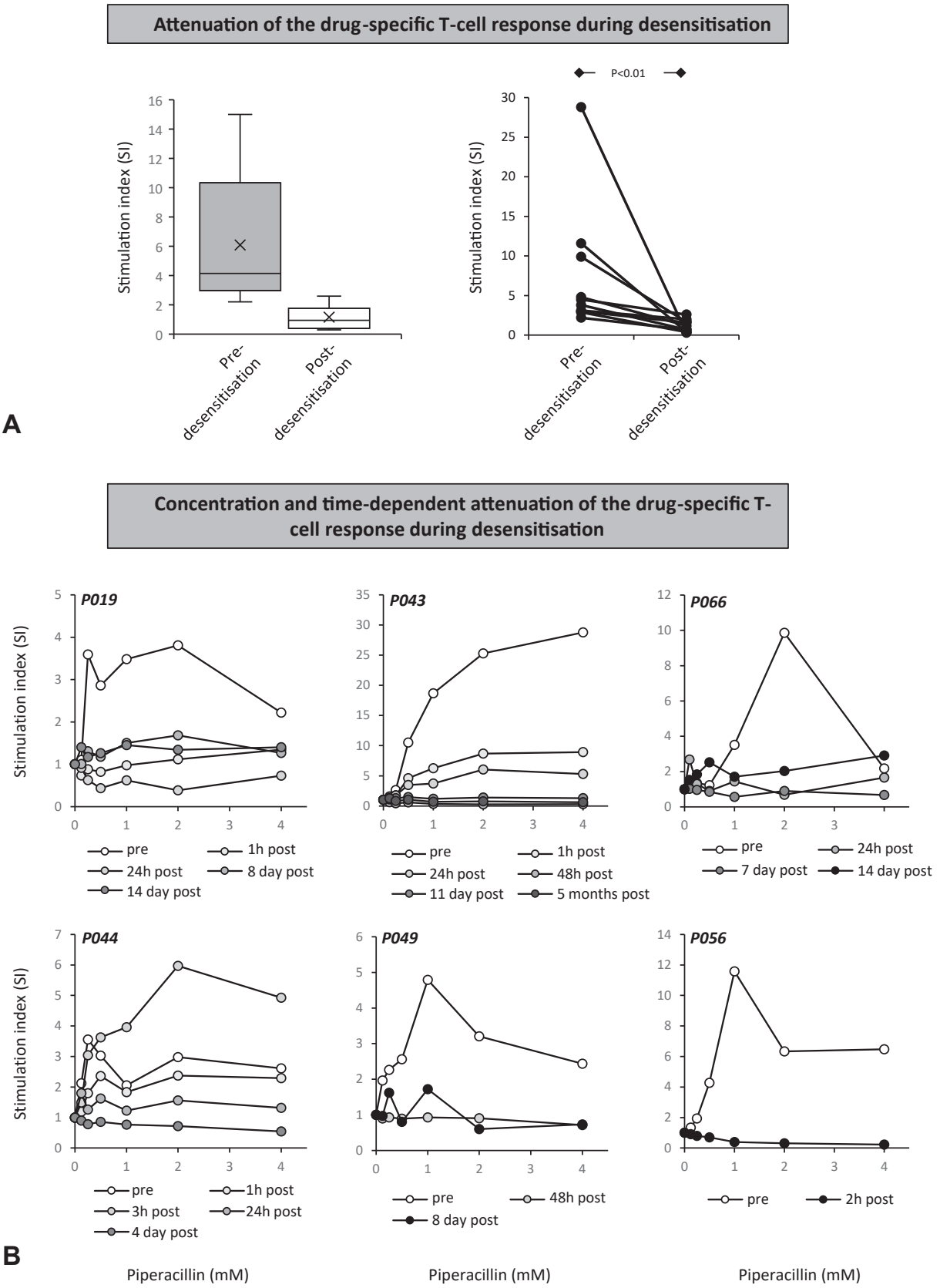


FIGURE 3. Positive piperacillin lymphocyte transformation test (LTT) assessment in tolerant patients. Peripheral blood mononuclear cells were incubated with titrated concentrations of piperacillin for 5 days in a 96-well *U*-bottomed plate (37°C; 5% CO_2). R9 medium served as a negative control. [^{3}H]Thymidine was added for 16 hours and proliferation was assessed by scintillation counting. (**A**) Positive piperacillin LTT in patients who subsequently tolerated the drug. The LTT was negative after treatment. P005 tolerated eight piperacillin-tazobactam treatment courses during follow-up. P076 tolerated three piperacillin-tazobactam treatment courses during follow-up and then developed hypersensitivity during the fourth course (see Tables E1, I, and II). (**B**) Positive piperacillin LTT in baseline tolerant patients on recruitment or with piperacillin-tazobactam treatment during the 3-year interventional phase of the study. All three patients (P072, P074, and P080) developed hypersensitivity with piperacillin-tazobactam treatment during the observational phase.

On recruitment to the study, PBMCs from patient 076 were activated with piperacillin in a dose-dependent manner. In contrast, the LTT was negative when blood was sampled 7 days after piperacillin-tazobactam treatment and on completion of the treatment course with no adverse event. Positive piperacillin LTTs, with an SI between 3 and 5 were recorded using PBMCs from patient P005 before and after the first course of piperacillin-tazobactam treatment. Seven months later, P005 tolerated an additional regular piperacillin-tazobactam treatment course, and the LTT was negative before and after treatment. In the 8-year observational period, these patients received 42 additional regular piperacillin-tazobactam treatment courses, with one hypersensitivity reaction reported (Table E1 and II, and Figure 1).

Three grade +/++ piperacillin LTTs were recorded in tolerant patients on recruitment (with one to three prestudy piperacillin-


Attenuation of the drug-specific T-cell response during desensitisation
Stimulation index (SI)
Pre-desensitisation
Post-desensitisation
P<0.01
A
Concentration and time-dependent attenuation of the drug-specific T-cell response during desensitisation
P019
P043
P066
P044
P049
P056
pre
1h post
24h post
8 day post
14 day post
48h post
11 day post
5 months post
7 day post
3h post
4 day post
2h post
Piperacillin (mM)
B

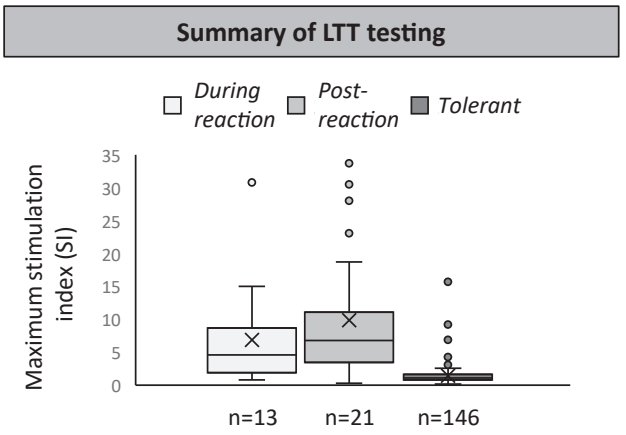


FIGURE 5. Summary of piperacillin lymphocyte transformation testing in tolerant patients and hypersensitive patients during the during the hypersensitivity reaction and after the reaction when symptoms subsided. Peripheral blood mononuclear cells were incubated with piperacillin for 5 days in a 96-well *U*-bottomed plate (37°C; 5% CO_2). R9 medium served as a negative control. [^{3}H]Thymidine was added for 16 hours and proliferation was assessed by scintillation counting. Data are presented as a box and whisker plot.

tazobactam treatment courses for P072, P074, and P080). In study, two patients were not exposed to additional piperacillin-tazobactam treatment. The third patient received a single course with a positive LTT recorded before and after treatment. All three patients received regular piperacillin-tazobactam treatment during the observational phase of the study, each of whom developed hypersensitivity. Figure 3, *B* shows LTT data from all three patients.

STUDY ARM 2: HYPERSENSITIVE PATIENTS RECRUITED FOR PIPERACILLIN-TAZOBACTAM DESENSITIZATION

Seventeen patients with a clinically diagnosed piperacillin-tazobactam hypersensitivity reaction were recruited to study arm 2 before a planned desensitization. Peripheral blood mononuclear cells from 15 patients were stimulated to proliferate in a concentration-dependent manner with piperacillin before desensitization. Ten patients progressed to desensitization and seven safely tolerated the procedure and 14-day piperacillin-tazobactam treatment. The desensitization procedure followed a standard protocol that involves a seven-step approach with 10-fold increases in concentration with each step until a therapeutic dose was achieved. Each step was given over 20 minutes and the total procedure lasted 2 hours 20 minutes.

The positive LTTs were graded as: n = 3, +; n = 6, ++; n = 2, +++; and n = 4, ++++. The piperacillin LTT was negative with PBMCs from two patients categorized clinically as hypersensitive. Both patients progressed to desensitization and tolerated piperacillin-tazobactam.

The strength of the piperacillin-specific proliferative response decreased during desensitization in all 10 patients with positive LTTs (mean maximum SI before and 1-3 days after desensitization [earliest post-desensitization time point analyzed], 6.8 ± 7.9; decreased to 1.2 ± 0.8; $P < .01$) (Figure 4 *A*). T cells remained unresponsive during treatment. Figure 4, *B* shows time- and concentration-dependent piperacillin-specific PBMC proliferative responses from six patients, five of whom (P019, 043, 049, 056, and 66) tolerated desensitization and 14-day piperacillin-tazobactam treatment.

Importantly, similar decreases in tetanus toxoid (employed as a positive control antigen) PBMC proliferation was not observed during or after piperacillin-tazobactam desensitization. Figure E2 (in this article's Online Repository at www.jaci-inpractice.org) shows tetanus toxoid LTT results from six desensitized patients (each with robust responses to tetanus toxoid) before, 1 and 24 hours after, and 1 to 8 days after desensitization.

During the observational phase of the study, eight of the desensitized originally LTT-positive patients tolerated 62 piperacillin-tazobactam courses through desensitization, four patients received no subsequent piperacillin-tazobactam, and three patients failed subsequent desensitization (Table III). The two LTT-negative patients tolerated seven subsequent piperacillin-tazobactam courses through desensitization with no adverse events noted.

Specific exceptional case descriptions

In patients P019 and P056, blood samples were obtained during desensitization, and the positive LTTs decreased from positive (SI 4 and 12, respectively) to negative (SI <2) in samples obtained before and 3 hours after desensitization, respectively. Blood was obtained from patient P019 at 1, 8, and 14 days after desensitization. The positive LTT decreased after 1 day and remained negative during piperacillin-tazobactam treatment. Patient P043, with a pre-desensitization piperacillin maximum LTT SI of 29, was sampled at 1, 24, and 48 hours, 11 days, and 5 months after desensitization. Attenuation of the piperacillin-specific proliferative response was time-dependent, with maximum SIs of 9 and 6 recorded after 1 and 24 hours, respectively. After 48 hours, a negative LTT was recorded, and a state of piperacillin tolerance was still observed 5 months after desensitization. Patient P044 failed the desensitization procedure, with a hypersensitivity reaction observed on day 19; maximum piperacillin PBMC proliferation increased from an SI of 3 to 6 during desensitization before negative LTTs were recorded 1 and 4 days after desensitization. When a blood sample was obtained 8 months after the failed desensitization, a grade ++ positive LTT was observed.

FIGURE 4. Attenuation of piperacillin-specific T-cell response during desensitization. (**A**) Peripheral blood mononuclear cells isolated from 10 hypersensitive patients before and after desensitization (sample analyzed 1 to 3 days after desensitization [earliest post-desensitization time point selected]) were used in the lymphocyte transformation test. Statistical analysis compares maximum piperacillin-specific peripheral blood mononuclear cell proliferative response before and after desensitization (Mann-Whitney test). (**B**) Time- and concentration-dependent assessment of piperacillin-specific lymphocyte transformation test responses during and after desensitization from six representative patients. Blood was sampled before desensitization and at various points during and after desensitization, depending on patient availability.

SUMMARY OF LTT RESULTS

Figure 5 displays results of LTTs conducted in study arms 1 and 2. The LTT had a sensitivity of 89% (positive response in hypersensitive patients) when used in patients with cystic fibrosis and a clinical diagnosis of piperacillin hypersensitivity. The real-world LTT sensitivity in this patient cohort is likely higher because two patients with piperacillin LTT-negative hypersensitivity who underwent successful desensitization likely received an incorrect diagnosis. Positive piperacillin LTTs were observed with blood sampled from hypersensitive patients at reaction diagnosis during a 14-day treatment course and when symptoms subsided. The strength of the maximum proliferative response was higher using samples collected after the adverse event (mean SI, 9.9 ± 9.6) compared with adverse event sampling (mean SI, 6.4 ± 7.9), but this did not reach statistical significance owing to the large interindividual variation. As expected, the maximum PBMC proliferative response observed with piperacillin in hypersensitive patients was significantly higher than the maximum observed with tolerant patients (mean SI, 1.5 ± 1.6; $P < .001$).

The specificity of the LTT (negative responses in tolerant patients) was 93%. Similar to the earlier discussion, the specificity should be higher (95%), because three tolerant patients with a positive LTT developed a hypersensitivity reaction when challenged with piperacillin-tazobactam.

When an SI cutoff of 5 or more (graded +++ in our system) is used to assign a positive LTT result, the sensitivity of the assay reduces to 50% (positive LTT in allergic patients) and the specificity is 98% (negative LTT in tolerant patients). This cutoff provides useful information on the performance of the assay, but it clearly mislabels several allergic patients. Furthermore, in three tolerant patients a positive LTT response was recorded.

DISCUSSION

The LTT is a simple *in vitro* assay used to diagnose non-immediate drug hypersensitivity reactions.[17-21] Over recent years ELIspot and flow cytometry assays have been used alongside the LTT to measure secreted cytokines and cell activation markers for improved diagnostic sensitivity in patients with severe forms of cutaneous hypersensitivity.[21,24-26] However, in patients with milder forms of drug hypersensitivity (eg, maculopapular eruptions) there is no consensus as to whether such assays offer an improvement over the LTT. Glassner et al[27] reported that antibiotics unexpectedly stimulated cytokine release from PBMC of non-hypersensitive patients. In our laboratory we have used the traditional [^{3}H]thymidine-based LTT and a panel of cytokine ELIspots to study PBMC responses to piperacillin in hypersensitive patients with cystic fibrosis.[9,22,28,29] In patients with moderate to strong piperacillin LTT responses, cytokine secretion is also detected. In patients with weakly positive LTT responses, it is often difficult to differentiate drug-specific cytokine release from background responses in medium control wells. For this reason, and because of the high sensitivity of the LTT for a piperacillin hypersensitivity diagnosis in patients with cystic fibrosis,[22] we choose to focus on the LTT in the current study to monitor the drug-specific immune response in (1) baseline tolerant patients exposed to multiple piperacillin-tazobactam treatment courses, and (2) hypersensitive patients subjected to desensitization. Our LTT analysis was restricted to piperacillin. We did not assess tazobactam and commercial piperacillin-tazobactam because our previous analysis of over 50 patients with suspected piperacillin-tazobactam hypersensitivity revealed only one tazobactam-positive LTT,[30] and because pure piperacillin and commercial piperacillin-tazobactam display identical LTT dose response curves when one controls for the piperacillin concentration (unpublished data).

Because laboratory-specific cutoffs are sometimes used to record a positive LTT, the initial component of our study was to establish a robust LTT grading system. Previous studies suggested that an SI of 2 or greater (double the proliferation in drug-treated wells compared with medium-treated wells) represents a positive reading and indicates drug-responsive T cells are detectable in patients' peripheral blood. In contrast, for β-lactam antibiotics such as piperacillin, an SI of 3 or greater may more accurately represent a positive result, because SI readings of 2 to 3 have been observed in tolerant control subjects.[17,18] Taking this into account, and considering our previous experience interpreting β-lactam antibiotic LTT results, we denoted a + positive LTT as an SI of 2 or greater, or less than 3, displaying dose-dependency with at least three drug concentrations. This categorization excluded single-dose readings with an SI of 2 or greater, or less than 3, sometimes caused by a single well of triplicate cultures being unexpectedly high, falsely becoming recorded as a positive LTT. Higher responses were recorded as ++ to ++++ with increasing SI.

On completion of the 8-year observational phase, 58 patients in study arm 1 were administered 611 piperacillin-tazobactam treatment courses. Twenty hypersensitivity reactions were reported (11 during the interventional phase and nine during the observational phase), with an average of 7.7 ± 5.0 piperacillin-tazobactam courses administered before the onset of reaction. The LTT assays were performed with PBMCs collected from the 11 interventional phase hypersensitive patients at diagnosis during the 14-day treatment course. This was important because normal advice is to conduct LTTs several weeks to months after symptom resolution, with little mechanistic justification or consensus. For example, Cabanas et al[31] reported the LTT sensitivity in patients with drug rash with eosinophilia and systemic symptoms was 73% with samples taken in the recovery phase, but only 40% in the acute phase, whereas Kano et al[32] reported that positive LTT responses were detected in patients with maculopapular drug eruptions during acute reactions and shortly after, but that these responses declined rapidly after patients recovered. This contrasts with our previous experience with piperacillin hypersensitivity in patients with cystic fibrosis, in whom positive LTTs were detectable more than 10 years after the adverse reaction.[22,28] In the current study, the LTT was positive in eight of 11 patients with PBMCs isolated during acute reaction blood sampling. Additional samples were taken 1 to 3 months after reaction resolution from patients with negative LTTs, and positive readings were obtained in two of three patients. The patient who recorded negative LTTs at both time points developed facial swelling 1 day after drug exposure as the only symptom of hypersensitivity. The patient was never re-exposed to piperacillin-tazobactam.

In total, 149 piperacillin LTTs were conducted before and after drug treatment in 47 patients classified clinically as drug tolerant throughout the 3-year interventional phase of the study. We recorded 139 negative LTT across 39 patients; this patient group tolerated 184 additional regular piperacillin-tazobactam

treatment courses during the 8-year observational phase of the study, with five hypersensitivity reactions recorded. Five tolerant patients recorded one or more positive LTT results and went on to tolerate regular piperacillin-tazobactam safely during the 3-year interventional phase of the study, with negative LTT readings observed after the treatment course and during the 8-year observational phase. These data indicate that piperacillin-specific T cells are generated at the time of drug exposure in a small number of individuals without the development of hypersensitivity symptoms, and that some form of tolerance is induced through subsequent piperacillin-tazobactam treatment. Additional studies are therefore needed to determine why the development of drug-specific T cells does not lead to hypersensitivity in this small cohort and pathways of tolerance induction. In mouse models of contact hypersensitivity, high doses of hapten-modified syngeneic cellular protein have been shown to induce a long-lasting and specific immunosuppressive effect.[33] It will be interesting to see whether exposure to different levels of piperacillin hapten alter the nature of the induced T-cell response and the clinical outcome after piperacillin-tazobactam use.

Positive piperacillin LTTs were also recorded in three tolerant patients either on recruitment or at the end of a piperacillin-tazobactam treatment course. Each patient developed hypersensitivity when exposed to piperacillin-tazobactam during the observational phase of the study. These data indicate that it might be possible to predict hypersensitivity reactions through real-time LTT testing, which would benefit future patient management by directing patients toward desensitization and/or alternative antibiotic treatments.

Although desensitization protocols for immediate IgE-mediated drug reactions are routine, less emphasis is placed on their equivalent use during nonimmediate reactions.[34-36] This is partly due to apprehension among medical professionals to administer a drug to a patient whom they know is sensitized, particularly when desensitization failure rates are typically 25%.[37] In a previous retrospective study from the Leeds cystic fibrosis group reviewing 275 desensitization procedures in 42 patients with a range of nonimmediate reactions to six commonly used antibiotics, 91% of procedures were successful, and the individual patient success rate for piperacillin-tazobactam was 55%.[14] Although these data indicate that desensitization is a safe and rapid procedure for readministering a drug to a hypersensitive patient with cystic fibrosis, a lack of mechanistic understanding remains regarding the immune regulatory processes that develop during drug treatment. In fact, it has been argued that the success of desensitization protocols in nonimmediate hypersensitivity might relate to an incorrect allergy diagnosis (World Allergy Congress, Istanbul, Turkey, 2022). For this reason, study arm 2 recruited 17 piperacillin-hypersensitive patients before planned desensitization to determine whether the procedure was successful in LTT-positive patients and the effect of desensitization on the piperacillin-specific T-cell response.

A positive piperacillin LTT was recorded in 15 patients; 10 progressed to desensitization, with seven safely tolerating the piperacillin-tazobactam desensitization and a 14-day treatment. During the observational phase of the study, eight patients received and tolerated 62 piperacillin-tazobactam courses through desensitization. The drug-specific T-cell response was reduced rapidly during successful desensitization and the LTT remained subdued during the 14-day piperacillin-tazobactam treatment course. Although further research is needed to define the duration of tolerance induction, the LTT in one patient remained negative when blood was sampled 5 months after desensitization. These data showing that desensitization induces tolerance rather than a temporary state of nonresponsiveness indicate that desensitization might no longer be necessary before future piperacillin-tazobactam treatment. It might also be possible to use the LTT to determine the future pathway for piperacillin-tazobactam treatment, with positive and negative results indicating desensitization or regular treatment, respectively. The rate of decrease in the piperacillin-specific LTT in the failed desensitization group was slower, which may provide a time frame for *in vivo* activation of T cells and the development of clinical symptoms of the adverse event. Thus, further research is needed to optimize the duration of the desensitization procedure, characterize effector and regulatory immune responses, and monitor levels of the drug protein adducts formed.

Potential mechanisms of tolerance induction during desensitization for immediate reactions are based on the antigen-specific induction of mast cell and basophil inactivity, including the downregulation of signaling molecules required for immune activation, the internalization of cross-linked antigen receptors, and reshaping of the effector T-cell response.[38-44] In a mouse model of passive anaphylaxis, Ang et al[45] reported that reduced degranulation responses in desensitized mast cells might arise from aberrant actin remodeling. In comparison, little is known about the mechanisms of desensitization for nonimmediate reactions, and only a handful of case reports examined T-cell activation before and after desensitization. Data from a single patient with allopurinol-induced fixed drug eruption indicated an inverse relationship between effector and Tregs after desensitization, with a reduction in $CD8^+$ T cells and increase in classical Tregs ($CD4^+$ $CD25^+$ T cells) in the lesion itself, highlighting a potential role for immune Tregs.[46] Another study indicated the utility of carboxyfluorescein succinimidyl ester (CFSE) staining in a donor with a negative LTT to monitor T-cell proliferation during desensitization to phenytoin. Those authors found a reduction in the number of $CFSE^{low}$-stained $CD4^+$ T cells after desensitization indicative of a reduction in proliferative response.[47] We previously showed that coinhibitory receptors such as programmed cell death protein 1, cytotoxic T-lymphocyte associated protein 4, and T-cell immunoglobulin and mucin domain 3 are rapidly upregulated during drug-specific T-cell activation[48,49]; hence, the outcome of desensitization (success or failure) might relate to different kinetics for increased coinhibitory receptor expression compared with the timings for effector T-cell tissue accumulation and activation.

Our study had several limitations, some of which will be addressed by future research. First, although our study is the largest to date to monitor PBMC proliferative responses prospectively in patients with drug hypersensitivity, the number of patients recruited to certain arms was relatively small. Second, the number of treatment courses and the number of samples obtained per patient were highly variable. Third, the number of blood samples collected more than 1 month after desensitization was heterogeneous. Thus, results from this component of the study should be regarded as preliminary. Finally, the LTT relies on [^{3}H]thymidine, which can be a limitation in other centers. Future studies should therefore compare other potential alternatives such as CFSE staining to detect proliferating T cells.

Our detailed assessment of piperacillin-tolerant and hypersensitive patients illustrates that the LTT has high sensitivity and specificity. Thus, use of the LTT should be considered in hypersensitive patients with limited treatment alternatives, to inform patient management. Disappearance of the drug-specific PBMC proliferative response during desensitization provides evidence that the procedure induces a state of immune tolerance. Future research should be directed toward understanding the pathways involved.

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ONLINE REPOSITORY

TABLE E1. Baseline characteristics and post-study allergy status of tolerant patients in study-arm 1

Patient*	Age/sex	FEV_1 (%)	Body mass index	Allergies at baseline	Piperacillin courses before study	Subsequent allergies after study (piperacillin reactions highlighted)
Piperacillin-tazobactam tolerant patients with negative LTT†						
002	20/M	31	15.3	NKDA	7	Meropenem: immediate reaction (tight throat/ wheeze within 1 h)
003	22/F	23	21.2	Ceftazidime (2010) delayed urticarial reaction	1	Tolerated two courses of piperacillin-tazobactam and then on third course developed drug fever and facial swelling on day 5. Later failed desensitization.
004	36/F	36	20.8	Meropenem (2010) tight chest and urticaria within 1 h	6	No subsequent piperacillin-tazobactam exposure
006	38/M	80	22.1	NKDA	1	Received seven courses of piperacillin-tazobactam after recruitment, no allergy
010	21/M	92	20.2	NKDA	2	Received six courses of piperacillin-tazobactam with no reactions. Colomycin (2016) urticaria; ceftazidime (2017) maculopapular rash
016	30/M	86	23.4	Ceftazidime (2009) urticaria and lip swelling	6	Received six courses of piperacillin-tazobactam after recruitment, no allergy
017	33/M	54	22.5	Ceftazidime (2007) urticaria and chest tightness	15	Received 21 courses of piperacillin-tazobactam after recruitment, no allergy

021	27/F	57	25.2	Ceftazidime (2007) maculopapular rash Piperacillin-tazobactam (2007 and 2012) vomiting	5	No piperacillin-tazobactam exposure after course of treatment at recruitment
022	26/F	33	16.3	Ceftazidime (2012) maculopapular rash	5	Received one course of piperacillin-tazobactam after recruitment, no allergy
023	55/F	53	22.5	NKDA	5	Received four courses of piperacillin-tazobactam after recruitment, no allergy
025	24/M	59	20.8	Ceftazidime (2006) maculopapular rash Aztreonam (2007) maculopapular rash	8	Tolerated two courses without reaction; on third course developed maculopapular rash and swollen hand on day 2
026	19/M	85	26.1	NKDA	1	Received two courses of piperacillin-tazobactam after recruitment, no allergy
027	31/M	40	24.9	Ceftazidime (2004) maculopapular rash Cotrimoxazole (2007) maculopapular rash	15	Received one course of piperacillin-tazobactam after recruitment, no allergy
028	33/M	28	21.4	NKDA	6	Received four courses of piperacillin-tazobactam after recruitment without reaction; on fifth course reported severe arthralgia
029	34/F	72	20.5	NKDA	5	Received 17 courses of piperacillin-tazobactam after recruitment, no allergy
030	32/F	66	20.1	NKDA	1	Received three courses of piperacillin-tazobactam after recruitment, no allergy
031	32/F	23	19.8	Ceftazidime (2008) maculopapular rash Aztreonam (2009) maculopapular rash	8	Received five courses of piperacillin-tazobactam after recruitment, no allergy

(continued)

TABLE E1. *(Continued)*

Patient*	Age/sex	FEV_1 (%)	Body mass index	Allergies at baseline	Piperacillin courses before study	Subsequent allergies after study (piperacillin reactions highlighted)
035	24/M	107	35	NKDA	0	Received one course of piperacillin-tazobactam after recruitment, no allergy
037	24/F	87	28.1	Ceftazidime (2012) delayed urticarial rash Aztreonam (2012) maculopapular rash	2	Received seven courses of piperacillin-tazobactam after recruitment, no allergy
038	24/M	73	20.3	Ceftazidime (2008) urticaria and angioedema Aztreonam (2013) urticaria	0	Received two courses of piperacillin-tazobactam after recruitment, no allergy
039	21/M	65	19.5	NKDA	4	Received four courses of piperacillin-tazobactam after recruitment, no allergy
040	17/F	47	18.2	Ceftazidime (2011) urticaria	1	Received five courses of piperacillin-tazobactam without reaction; on sixth course developed maculopapular rash and red eyes on day 3
041	39/F	59	20.6	Meropenem (2002) anaphylaxis Ceftazidime (2005) maculopapular rash Aztreonam (2007) joint pains	32	Received five courses of piperacillin-tazobactam after recruitment, no allergy
042	28/M	68	24.85	Ceftazidime (2010) delayed urticarial rash	4	Received four courses of piperacillin-tazobactam after recruitment, no allergy
045	66/M	37	26.4	NKDA	1	Received one course of piperacillin-tazobactam after recruitment, no allergy recorded

046	30/F	53	20.7	Ceftazidime (2010) maculopapular rash Aztreonam (2010) maculopapular rash Piperacillin-tazobactam (2011) drug fever	4	Received nine courses of piperacillin-tazobactam after recruitment; on ninth course developed drug fever and swollen eyes (note similar mild reaction in 2011)
047	51/M	58	25.6	Vancomycin (2008) urticarial rash Meropenem (2010) urticaria	12	Received six courses of piperacillin-tazobactam after recruitment, no allergy recorded
048	19/F	68	23.4	NKDA	1	Received three courses of piperacillin-tazobactam after recruitment; on third course had drug fever and arthralgia on day 6
054	18/F	28	15.9	NKDA	10	Received four courses of piperacillin-tazobactam after recruitment, no allergy
060	21/M	37	20.3	Ciprofloxacin (2007) maculopapular rash	3	Received one course of piperacillin-tazobactam after recruitment, no allergy
062	41/M	25	23.2	NKDA	2	Received 11 courses of piperacillin-tazobactam after recruitment, no allergy
063	28/M	27	21.6	NKDA	2	Received nine courses of piperacillin-tazobactam after recruitment, no allergy
065	23/F	69	18.2	Ceftazidime (2001) maculopapular rash	2	Received three courses of piperacillin-tazobactam after recruitment, no allergy
068	18/F	90	16.9	NKDA	1	Received three courses of piperacillin-tazobactam after recruitment, no allergy
070	48/F	63	24.1	NKDA	1	Received two courses of piperacillin-tazobactam after recruitment, no allergy

(continued)

TABLE E1. *(Continued)*

Patient*	Age/sex	FEV_1 (%)	Body mass index	Allergies at baseline	Piperacillin courses before study	Subsequent allergies after study (piperacillin reactions highlighted)
071	35/M	45	28.3	NKDA	2	Received two courses of piperacillin-tazobactam after recruitment, no allergy
075	34/F	52	22.3	Amoxicillin (2009) urticarial rash	3	Received four courses of piperacillin-tazobactam after recruitment, no allergy
082	20/M	22	16.5	NKDA	11	Received 13 courses of piperacillin-tazobactam after recruitment, no allergy
085	29/F	80	16.4	Ceftazidime (2007) maculopapular rash	10	Received seven courses of piperacillin-tazobactam after recruitment, no allergy recorded
Piperacillin-tazobactam tolerant patients with positive LTT recorded, which weakened during treatment‡						
005	39/F	48	23.3	Aztreonam (2008) elevated liver function Meropenem (2000) headache and drug fever	10	Received eight courses of piperacillin-tazobactam after recruitment, no allergy recorded
012	26/M	26	20.1	Colomycin (2012) headache	3	Received six courses of piperacillin-tazobactam after recruitment, no allergy recorded
018	30/M	83	30.8	NKDA	1	Received four courses of piperacillin-tazobactam after recruitment, no allergy recorded
052	21/F	83	19.5	NKDA	2	Received 21 courses of piperacillin-tazobactam after recruitment, no allergy
076	25/M	35	20.9	NKDA	4	Tolerated three courses after recruitment then developed urticarial rash to piperacillin-tazobactam (2016) on fourth course at day 7

Piperacillin-tazobactam tolerant patients with positive LTT during or after treatment with no further piperacillin-tazobactam treatment during study§						
072[4]	18/F	37	19.2	Cefaclor (2011) maculopapular rash Posaconazole (2014) maculopapular rash	3	Reaction to piperacillin-tazobactam while receiving course after recruitment; facial swelling and fevers on day 3
074	24/F	50	19.5	Meropenem (2008) urticarial rash	2	Tolerated one course after recruitment and then delayed urticarial rash to piperacillin-tazobactam on day 4 (2016)
080	29/F	76	18.9	NKDA	2	Tolerated seven courses and then developed maculopapular rash to piperacillin-tazobactam (2021) on eighth course on day 4

NKDA, no known drug allergy; *LTT*, lymphocyte transformation test.
*Patients 7, 36, 51, 53, 59, 67, 69, 78, 79, and 84 were excluded from the study (and Tables I, II, and III) because samples for LTT were not obtained.
†n = 39. After the study, patients received 184 piperacillin-tazobactam treatment courses. Five reactions were noted (13% of patients).
‡n = 5. After the study, patients received 43 piperacillin-tazobactam treatment courses. One reaction was noted (20% of patients).
§n = 3. After the study, all patients received piperacillin-tazobactam treatment courses and all reported reactions (100% of patients).

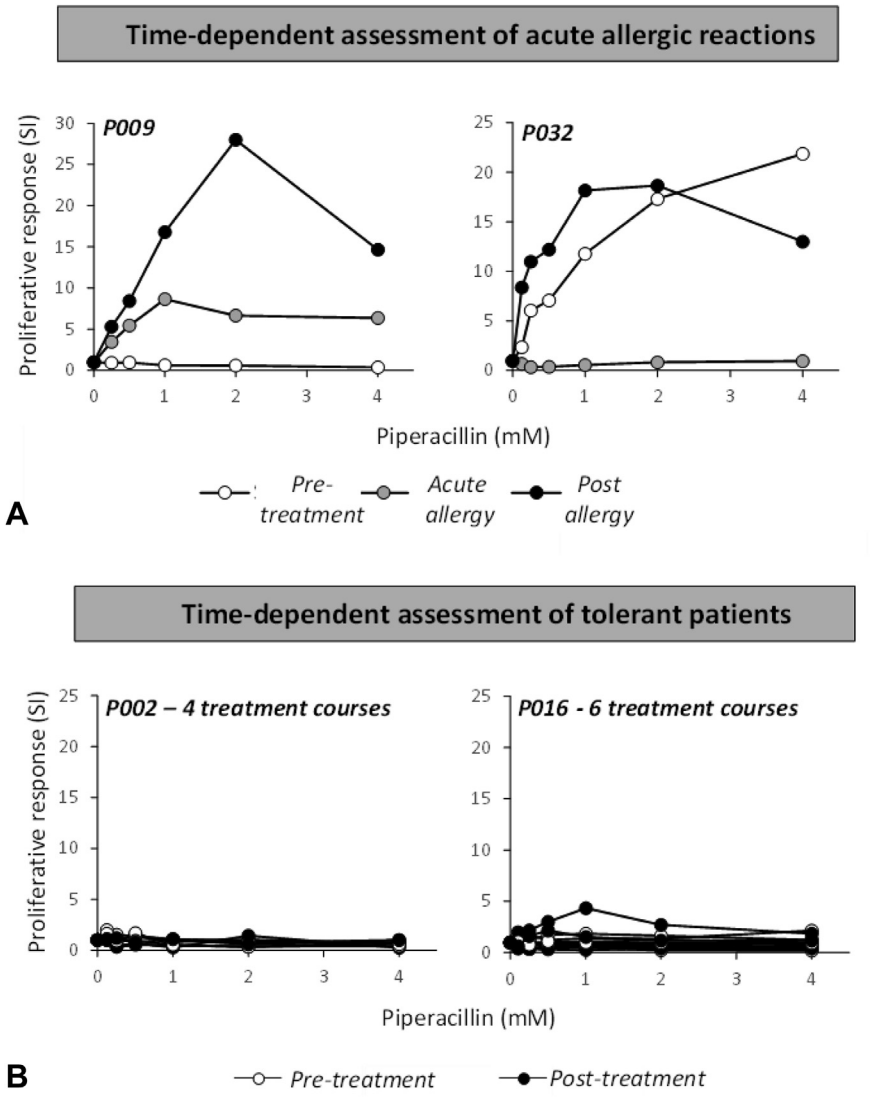

FIGURE E1. Time-dependent piperacillin lymphocyte transformation test assessment of (**A**) acute hypersensitivity reactions and (**B**) tolerant patients. Peripheral blood mononuclear cells were incubated with titrated concentrations of piperacillin for 5 days in a 96-well *U*-bottomed plate (37°C; 5% CO_2). R9 medium served as a negative control. [^{3}H]Thymidine was added for 16 hours and proliferation was assessed by scintillation counting. (A) Two patients developed hypersensitivity in the study. P032 recorded a positive lymphocyte transformation test on recruitment to the study despite tolerating previous piperacillin-tazobactam treatment courses. (B) Two patients tolerated multiple piperacillin-tazobactam treatment courses. *SI*, stimulation index.

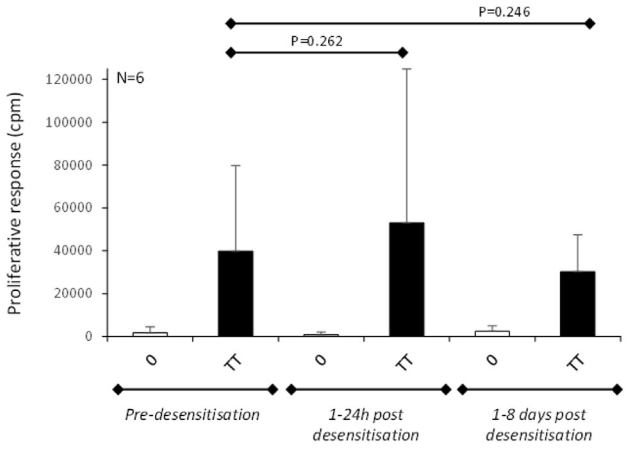

FIGURE E2. Stable irrelevant antigen (tetanus toxoid)-specific T-cell responses during desensitization. Peripheral blood mononuclear cells isolated from six hypersensitive patients before and after desensitization were incubated with tetanus toxoid for 5 days in a 96-well *U*-bottomed plate (37°C; 5% CO_2). R9 medium served as a negative control. [^{3}H]Thymidine was added for 16 hours and proliferation was assessed by scintillation counting. Post-desensitization results are separated according to the time of blood sampling (1-24 h and 1-8 days after desensitization). Statistical analysis compared maximum tetanus toxoid-specific peripheral blood mononuclear cell proliferative response before and after desensitization (Mann-Whitney test). *cpm*, counts per minute.