

Clinical usefulness of patch and challenge tests in the diagnosis of cell-mediated allergy to betalactams

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Background: Literature reports dealing with cell-mediated allergy to betalactams have appeared with increasing frequency in the last years.

Objective: To evaluate patients with such reactions and to identify cross-reactivities among betalactams in order to provide safe guidelines for their further clinical management.

Methods: Thirty consecutive subjects with cell-mediated allergy to betalactams (history of adverse reactions to these antibiotics; serum total IgE within the normal range; absence of serum specific IgE antibodies to penicillin G and V, amoxicillin, and ampicillin; negative skin tests with a wide pattern of betalactam preparations; and positive patch-test to at least one betalactam antigenic determinant) were investigated.

The subjects admitted to the study were patch tested with a wide variety of betalactam preparations in order to identify alternative molecules tolerated by the patient. To better evaluate the cross-reactivity pattern, tolerance challenges with patch-negative betalactams were also performed in each subject.

Results: Both specific IgE and skin tests were negative in all patients. The skin biopsies performed on the positive patch-tested area in four patients showed a clear T-lymphocyte, CD4⁺-type infiltrate, thus definitely proving the occurrence of a cell-mediated response.

A total of 44 adverse reactions (mean: 1.47 episodes for each patient) were reported in history, with a mean interval of 15 hours after betalactam administration. The reported symptoms were mainly cutaneous (maculo-papular rash and urticaria) and the responsible drugs were chiefly aminopenicillins (86.4% of cases) and penicillin G (9.1%).

We were able to identify three separate groups of patients on the basis of clinical history, patch-test, and tolerance challenge pattern:

- allergy to the side chain of aminopenicillins in 16 patients (53.3%);
- allergy to the thiazolidine ring in 3 patients (10.0%);
- undetermined specificity in the remainder 11 patients (36.7%).

Cross-reactivity among different betalactam molecules (revealed by positive tolerance tests performed with patch-negative betalactams) was found in 4.8% of cases only (23.3% of all investigated patients). This fact demonstrates a very high (95.2%) predictive value of a negative patch-test in excluding the occurrence of a cross-reactivity. The mis-match between patch and tolerance tests was observed in 3 out of 178 cases only (1.7% of cases, 10.5% of patients) in groups A and B, and in as much as 12.2% of cases (45.5% of subjects) in group C ($P < .05$).

Conclusions: Delayed allergy to betalactams (mainly to aminopenicillins) may be exerted by a cell-mediated response. Patch tests and tolerance challenges are extremely useful and safe for diagnosis and further clinical treatment of these patients, helping to identify safe alternative betalactam molecules that could be successfully tolerated by the allergic subjects.

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INTRODUCTION

Four different groups of antibiotics, all sharing a beta-lactam ring (BLR) in their basic chemical structure (Fig 1), are currently referred to as betalactams:

- *Penicillins* have a thiazolidine ring (THIAZ) in addition to BLR, and a side chain (SC) linked to BLR through the NH_2 group on C6.
- *Cephalosporins* hold a dihydrothiazinic ring instead of THIAZ, and could present up to three different SC: on C7 of BLR, on C3 and C4 of the dihydrothiazinic ring.
- *Carbapenems* do not have THIAZ because in position 1 there is a C instead of a S.
- *Monobactams* have a monocyclic basic structure, represented by BLR alone, to which up to three SC could link.

The chemical structure of betalactam molecules is of great importance in determining their antigenicity. In the past, penicillin was thought to act as an antigen on the whole;^{1,2} however, further studies³⁻⁹ have demonstrated that single parts of the molecule may act as antigens. Studies on IgE-mediated allergy,³ further confirmed by several clinical and immunologic reports, showed that there are at least three different epitopes in the penicilloyl groups. They consist in the "new antigenic determinant" (NAD, resulting from the opening of the BLR and conjugation to endogenous proteins), thia-

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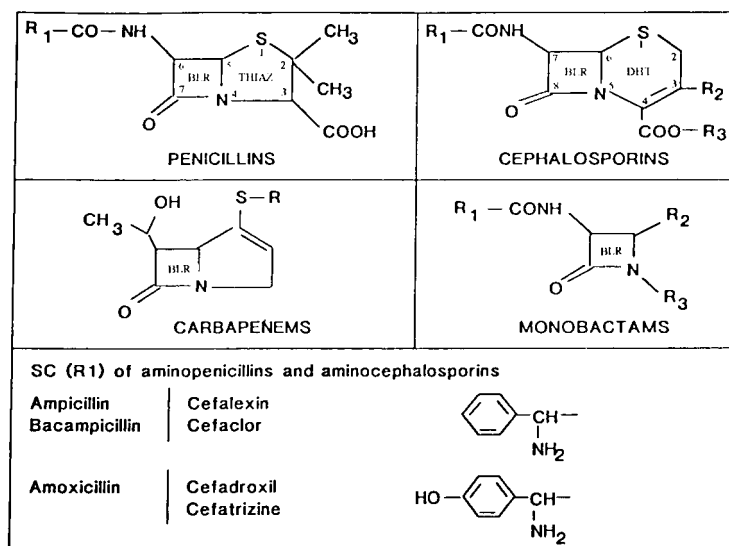


Figure 1. Basic chemical structure of betalactams. BLR = betalactam ring, THIAZ = thiazolidine ring, DHT = dihydrothiazine ring, and R, R1, R2, and R3 = side chains.

olidine ring and side chains, which may be involved in different allergic responses of various patients.

The antigenic importance of the SC of aminopenicillins (amoxicillin plus ampicillin and its ester compounds), which share an α -NH₂ group on BLR with a group of cephalosporins referred to as aminocephalosporins (cefaalexin, cefachlor, cefadroxil, cefatrizine, cefradine, and cefroxadine), has been particularly stressed in the last years^{4,10-15} as well as the role of THIAZ.^{7,16}

Allergy to betalactams has been thought traditionally to be associated with a type I, IgE-mediated hypersensitivity. During these last years several reports of systemic reactions to betalactams due to a type IV, cell-mediated, hypersensitivity mechanism appeared in the literature.^{12,16-21} Such reactions are often revealed by cutaneous symptoms (a maculopapular rash being the most typical clinical manifestation),²² particularly after the administration of aminopenicillins. The clinical management of patients with allergic reactions to betalactams (both immediate and delayed type), and whether they could be further administered betalactams after a first allergic episode, still remain major problems in clinical practice.

The aim of the present study was to further ascertain the actual existence of a cell-mediated allergy to betalactam antibiotics, and to investigate the occurrence of cross-reactivity among various betalactam molecular configurations. Cross-reactivity was investigated by means of patch-tests and tolerance challenge tests with selected molecules (excluding those responsible for the reported adverse reaction, or showing a positive patch-test).

The combined results of patch and tolerance challenge tests may provide safe guidelines for the further clinical management of each patient, helping to identify alternative betalactam molecules that could be tolerated by the allergic subject. Further, the results of the tolerance tests allowed us to evaluate the value of a negative patch-test in predicting the patient's ability to tolerate other betalactam preparations.

METHODS

Patients with histories of adverse reactions to betalactams, normal serum total IgE levels, absence of specific IgE antibodies (CAP-FEIA, Pharmacia, Uppsala, Sweden) and negative skin tests for penicillin G and V, ampicillin, and amoxicillin were considered eligible for the study. To assess definitely the occurrence of a cell-mediated al-

lergy to betalactams, skin and patch tests with a wide pattern of betalactam preparations (Table 1) were performed in all patients. Only subjects with negative skin tests and a patch test positivity to at least one betalactam antigenic determinant (and therefore with proven cell-mediated allergy to betalactams) were further investigated.

Thirty consecutive patients (8 males and 22 females, aged from 17 to 63) over a period of 3 years matched the above mentioned admission criteria. All subjects were followed as out-patients at the Department of Allergology of Policlinico Gemelli, Rome, in a day-hospital regimen. An informed consent was obtained before each diagnostic test.

In all patients admitted to the study, matched tolerance challenge tests were carried out employing different betalactam molecules (excluding those responsible for the reported adverse reaction, or resulted positive to patch-tests).

In order to prove a definite cause-effect relationship between betalactam administration and reported symptoms, a provocative challenge with the drug(s) responsible for the reported adverse reactions (to which the patch-test was previously positive) was also performed in 11 patients who gave their informed consent.

Further details about the employed techniques are provided hereinafter:

Skin Tests

Skin tests were carried out on the volar surface of the forearm, first by prick and then, in case of negativity, intradermally, using 0.1 mL of the concentrations indicated in Table 1. The betalactams unavailable on the market for parenteral use couldn't be tested intradermally and were "prick by prick" tested only, using the tablet powder dissolved in saline (Table 1).

Evaluation was assessed after 20 minutes, and again 48 hours later, should any delayed reaction occur. Histamine as positive control and saline as negative control were employed. Reactions at least 3 mm > than controls in diameter for prick test

Table 1. Betalactams Used for in Vivo Tests

Drug	Skin Test Concentrations	Tolerance Challenges		
		R.A.	D I (mg)	D II (mg)
Penicillin G	0.2–2000 I.U./mL	im	0.25–2.5–25 KU.I.	250–250–500 KU.I.
Penicillin V	100.000 IU/mL	os	0.25–2.5–25 KU.I.	250–250–500 KU.I.
Ampicillin	1 mg/mL (2.86 mM/L)	os	5–10–15–20	50–100–150–200
Amoxicillin	1 mg/mL (2.74 mM/L)	os	5–10–15–20	50–100–150–200
Bacampicillin	90 mg/mL (193.3 mM/L)*	os	8–16–24–32	80–160–240–320
Piperacillin	5 mg/mL (9.66 mM/L)	im	20–40–80	160–320–520
Mezlocillin	2.5 mg/mL (4.63 mM/L)	im	20–40–80	160–320–520
Ticarcillin	6 mg/mL (14.0 mM/L)	im	20–40–80	160–320–520
Imipenem	2.5 mg/mL (8.38 mM/L)	im	10–20–40	80–160–260
Aztreonam	3.3 mg/mL (7.60 mM/L)	im	10–20–40	80–160–260
Cefalotin	2.5 mg/mL (6.31 mM/L)	im	20–40–80	160–320–520
Cefamandole	2.5 mg/mL (5.41 mM/L)	im	20–40–80	160–320–520
Acetoxyethyl- cefuroxime	90 mg/mL (212.1 mM/L)*	os	0.05–0.5–5	100–150–250
Cefalexin	90 mg/mL (259.1 mM/L)*	os	0.05–0.5–5	100–150–250
Cefaclor	90 mg/mL (244.7 mM/L)*	os	0.05–0.5–5	100–150–250
Cefadroxil	90 mg/mL (247.7 mM/L)*	os	0.05–0.5–5	100–150–250
Cefatrizine	90 mg/mL (194.7 mM/L)*	os	0.05–0.5–5	100–150–250
Cefixime	90 mg/mL (198.5 mM/L)*	os	0.05–0.5–5	50–100–250
Ceftibuten	90 mg/mL (219.3 mM/L)*	os	0.05–0.5–5	50–100–250
Cefotaxime	2.5 mg/mL (5.49 mM/L)	im	20–40–80	160–320–520
Ceftriaxone	2.85 mg/mL (5.14 mM/L)	im	20–40–80	160–320–520

* 'prick by prick' test.

RA = route of administration, im = intramuscular, os = oral, D I = dose given on the first day, and D II = dose given on the second day.

and 5 mm for intradermal tests were considered positive.

Skin tests were also performed employing penicilloyl-polylysine (PPL Allergopen, Bracco, Italy, containing 35 µg/mL, utilizing undiluted and 1:10 solutions) and Minor Determinant Mixture (MDM Allergopen, Bracco, Italy, containing 500 µg benzilpenicillin and 600 µg benzilpenicilloate per milliliter, using 1:10 and 1:10,000 solutions of the commercial preparation).

Patch Tests

Patch tests were carried out using the same drugs as for skin tests (Table 1) in all patients as well as in a control group of 20 healthy subjects (8 males and 12 females, aged 17 to 56) who reported a history of penicillin treatments with no allergic reactions.

Patches were applied on the interscapular region (48 hours), and evaluated 48 and 72 hours after application according to Wilkinson.²² Commercially available self-adhesive anallergic gauze strips (Curatest-Lohmann; Neuwied, Germany) were used as supports.

We used 0.1 mL of undiluted commercial solutions for PPL and MDM, 5,000 I.U. in vaseline for penicillin G, and the concentration of 50 mg/mL in vaseline for all the remainder drugs.

In four patients who gave their full informed consent, the positivity of patch tests was further confirmed by a biopsy of the skin resulted positive to patch-test, 72 hours after application (see "NOTE" for further methodologic details).

Tolerance Challenge Tests

Tolerance challenge tests followed the general schedule reported in Table 1. Each challenge test was performed over two days, each day administering increasing doses of the drug at 45-minute intervals. The interval between the first and second day of each challenge was at least 72 hours. On the second day the complete given dose corresponded to the common daily therapeutic dose.

Provocative Challenge Tests

A total of 13 challenges with the drug(s) responsible for the reported adverse reaction (and showing a positive patch-test) were performed in 11 patients. The general schedule followed for provocative challenges was the same as for tolerance ones (Table 1). Provocative tests were carried out with extreme caution, immediately administering an adequate therapy (antihistamines and corticosteroids) as soon as first symptoms appeared.

NOTE: Cutaneous biopsies were taken by an 8-mm punch. They were snap-frozen in Tissue-Tek embedding solution (Miles Lab, Naperville, IL) with liquid nitrogen and stored at –80°C. For immunochemical staining, specimens were cryostat-sectioned at 4-µm intervals. These sections were thaw-mounted on polylysine hydrobromide-coated slides (Polyscience Inc, Warrington, PA) and allowed to air-dry for 3 hours.

All subsequent steps were performed at room temperature. Sections were fixed for 10 minutes in 100% acetone. Endogenous peroxidase activity was blocked by treating samples with chloroform for ten minutes. Then the slides were rehydrated in 0.05 M TRIS-buffered saline (TBS), pH 7.6. Sections were then incubated for 30 minutes in normal serum, followed by an incubation for 1 hour with the following monoclonal antibodies: anti-CD3, anti-CD4, anti-

CD8 (Becton Dickinson, Mountain View, CA) and anti-CD20 (Dako, Glostrup, Denmark).

Isotype-matched controls were employed. Staining was achieved by the avidin-biotin peroxidase technique (ABC ELITE detection system; Vector, Burlingame, CA). Hydrogen peroxide, normal goat blocking serum, biotinylated immunoglobulins, avidin-biotin complexes, and 3-amino-9-ethyl-ethyl-

carbazole substrate solutions were used according to the manufacturer. Sections were counterstained with toluidine blue 0.5% for two minutes, and then dehydrated in isopropyl alcohol and mounted in Permount. Evaluation using light microscope followed.

RESULTS

According to the selection criteria, patch tests (Table 2) were positive to

one or more betalactam molecules in all investigated subjects (being negative in all controls). Serum total IgE were within the normal range in all patients. Further, the specific IgE antibodies for penicillin G and V, amoxicillin, and ampicillin were negative as well as all the skin (prick and intradermal) tests.

Such a test pattern outlines the presence of a type IV (cell-mediated) hypersensitivity mechanism, which has

Table 2. Results of Patch and Challenge Tests

Patient Number	Patch Test +	Challenge +	Challenge –
Group A			
2	Am,Ax,B	—	PG,PV,Pi,M,I,A,Clx,Crx,Cxi,Ccl
5	Am,Ax,B	B*	PV,PG,M,I,A,Clx,Cdx,Crx,Cxi,Ccl
7	Am,Ax,B	—	PG,PV,Pi,I,A,Ti,Clx,Clt,Crx,Cxo
9	Am,Ax,B	B*	PV,Pi,M,I,A,Crx,Cdx,Clx,Cxi
11	Am,Ax,B	—	PG,PV,A,Clx,Cdx,Pi,Cxi,Cxo
13	Am,Ax,B	—	PV,Pi,Clx,Cdx,Cxi,Cft
14	Am,Ax,B	—	PV,PG,Pi,I,A,Ti,Clx,Cdx,Cft,Cxo
15	Am,Ax,B	—	PV,PG,Pi,I,A,Clx,Ctr,Cdx,Clt,Cft,Cxo
19	Am,Ax,B	—	PG,PV,I,Clx,Cdx,Cft
20	Am,Ax,B	B*	PV,PG,Pi,I,A,Ti,Clx,Cdx,Crx,Cft,Cxo
21	Am,Ax,B	—	PV,PG,Pi,M,I,A,Clx,Cdx,Ctr,Crx,Cxi
22	Am,Ax,B	B*	PG,PV,Pi,I,A,Clx,Ctr,Cxi,Cxo
25	Am,Ax,B	Am*,Ax*	PV,PG,Pi,I,A,Ti,Clx,Ctr,Crx,Cfm,Cxi,Ccl
26	Am,Ax,B	—	PV,Pi,I,A,Clx,Crx,Cft
27	Am,Ax,B	Am*,Clx	PG,PV,Pi,I,A,Ctr,Cfm,Cxi,Cxo,Ccl
28	Am,Ax,B	—	PG,PV,Pi,I,A,Ti,Clx,Cdx,Ctr,Cft,Cxo,Ccl
Group B			
1	PG,PV,Am,Ax,B,Pi,M,Ti	—	I,A,Clx,Ctr,Crx,Cxi,Cxo
18	Am,Ax,B,M	PG,M*,Ti	Pi,I,A,Clx,Crx,Cxi,Cxo
30	PG,PV,Am,Ax,B,Pi,M,Ti	—	I,A,Clx,Ctr,Crx,Cfm,Cxi,Cxo,Ccl
Group C			
3	PG,PV,Ti	—	Am,Ax,Pi,I,A,Clx,Crx,Cft,Cxo
4	PG,PV,Am,Ax,B,Pi,M,Cdx	Clx,Cdx*	I,A,Crx,Cft,Cxo
6	Am,Ax,B,M,Pi,Cdx,Ctr,Crx,Cfm	Am*,Pi*	PG,PV,I,A,Clx,Cxi,Cxo
8	Am,Ax,B	PG,PV	Pi,I,A,Clx,Cdx,Crx,Cxi,Cxo
10	Am,Ax,B,M,Ti,Crx	Cxi	PV,Cdx,Crx
12	PPL,PG,PV,Am,Ax,B,Pi,M,I,Ti,Clx,Clt,Cdx	—	A,Ctr,Cft,Ccl,Cxo,Cfm,Clx,Crx
16	Am,Ax,B,PG,PV	Clx,Cxo	I,A,Crx,Cft
17	PPL,MDM,PG,PV,Am,Ax,B,Pi	Am*	Pi,M,I,A,Clx,Cdx,Crx,Cxi,Cxo
23	Am,Ax,B,Pi,Clx	Clx*	PG,Cft
24	MDM,PG,PV,Am,Ax,B	—	Pi,I,A,Clx,Crx,Cft,Cxo
29	Am,Ax,B	PG,Pi,Clx	I,A,Cft

* Provocative challenge test performed in patients with positive patch-test.

Abbreviations: PG = penicillin G, PV = penicillin V, Am = ampicillin, AX = amoxicillin, B = bacampicillin, Pi = piperacillin, M = mezlocillin, I = imipenem, A = aztreonam, Ti = ticarcillin, Clx = cefalexin, Clt = cefalotin, Cdx = cefadroxil, Ctr = cefatrizine, Crx = acetoxymethyl-cefuroxime, Cfm = cefamandole, Cxi = cefixime, Cft = ceftibuten, Cxo = ceftriaxone, Cax = cefataxime, Cfx = ceftazidime, and Ccl = cefactor.

been confirmed by the results of the biopsies performed in four patients (cases n. 18, 25, 27, 29) on the skin resulted positive to patch-tests. The microscopical findings were similar in all cases, showing (Fig 2, patient n. 27)

a lymphocytic infiltrate around superficial and deep vascular plexi associated with epidermal spongiosis and edema of papillary dermis (Fig 2-A). Most cells within the infiltrate were T lymphocytes reacting with CD3 (Fig

2-B). There was a predominance of CD4+ cells as compared with CD8+ ones (Fig 2 C and D, respectively). Only single scattered cells were B lymphocytes as shown by CD20 positivity (Fig 2-E).

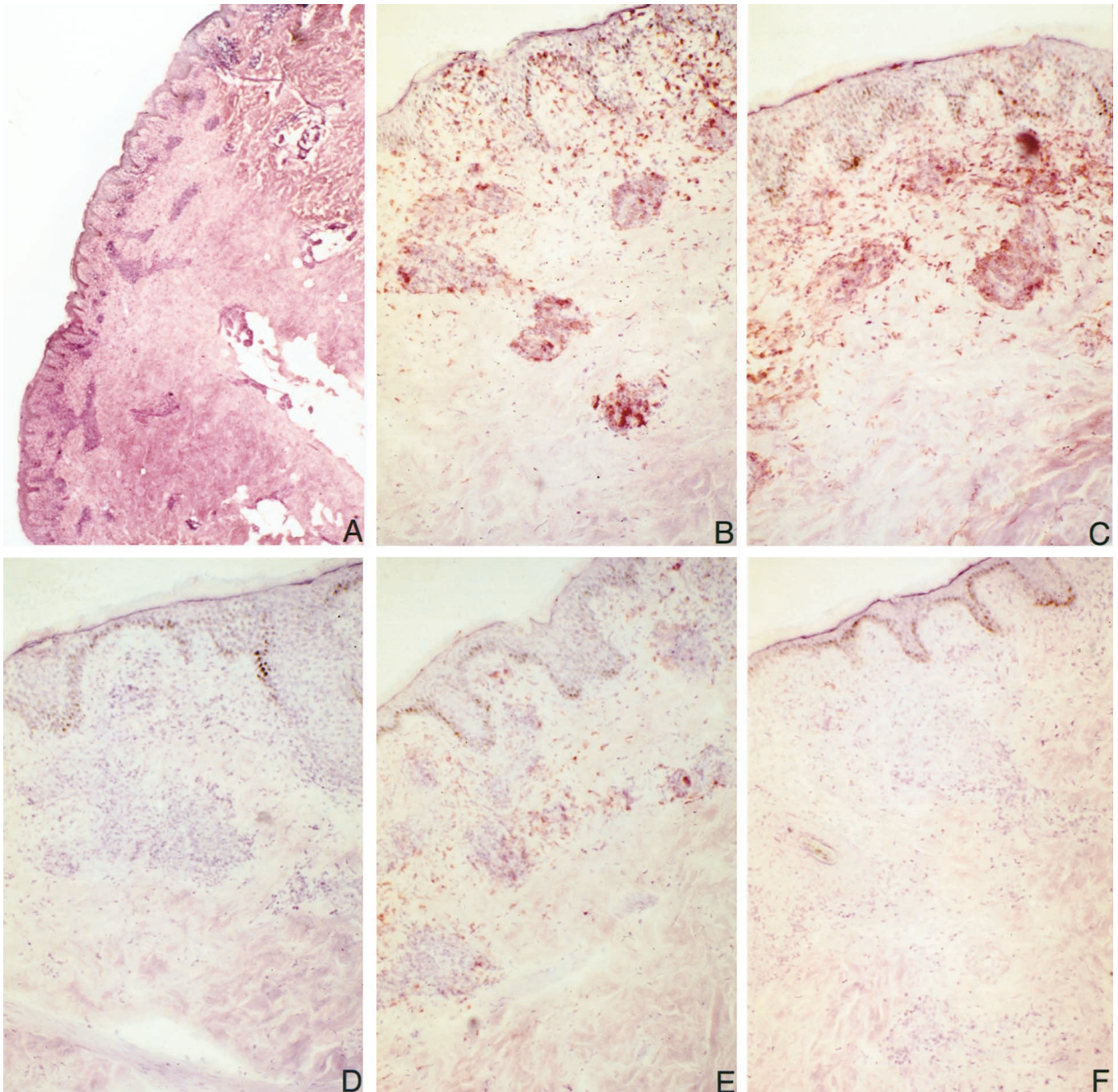


Figure 2. Skin biopsy specimens. (A) E and H, $\times 10$; (B) anti-CD3 used, $\times 10$; (C) anti-CD4 used, $\times 25$; (D) anti-CD8 used, $\times 25$; (E) anti-CD20 used, $\times 25$; and (F) negative control (neuron-specific enolase used) $\times 25$.

The patch tests with betalactams were negative in all control subjects.

The clinical data of the investigated patients are summarized in Table 3.

A total number of 44 adverse reactions was reported (mean $\pm$ 1.47 episodes for each patient), every single

patient referring one or two reactions. The mean interval between the last adverse reaction reported and clinical examination was 6 years (Table 3).

The responsible drugs were mainly aminopenicillins, with 38 episodes (86.4%) in 27 out of 30 patients (14

episodes with amoxicillin, 13 with ampicillin, and 11 with bacampicillin), and penicillin G with 4 episodes (9.1%) in 4 patients (13.3%). Piperacillin and cefalexin were responsible for only one episode (2.3% of cases).

Table 3. Clinical Data of the Investigated Patients

N.	Sex Age	Interval Between Reaction and Evaluation	N. of episodes	Drug	Interval Between Administration and Reaction	Symptoms
1	F-60	26 years	1	PG im	1 h	Erythema, dyspnoea, syncope
2	F-27	10 months	1	Ax os	48 h	Urticaria
3	M-33	25 years	1	PG contact	2 h	Angioedema
4	F-36	10 years	1	Am os	24 h	Maculopapular rash
5	M-20	10 months	1	B os	24 h	Maculopapular rash
6	F-26	11 years	1	Am os	48 h	Maculopapular rash
7	F-63	2 months	2	B os	12 h	Maculopapular rash
				B os	12 h	Maculopapular rash
8	M-57	1 month	2	Am os	12 h	Maculopapular rash
				B os	12 h	Maculopapular rash
9	F-69	3 months	2	Am os	7 h	Urticaria, angioedema
				Ax os	24 h	Urticaria, angioedema
10	F-32	7 years	1	Clx im	12 h	Maculopapular rash
11	F-44	10 years	2	Ax os	10 h	Pruritus, erythema
				Am os	12 h	Maculopapular rash
12	F-44	5 years	2	Am os	4 h	Maculopapular rash
				Ax os	1 h	Maculopapular rash
13	M-25	1 year	2	Ax os	72 h	Urticaria
				Ax os	12 h	Urticaria
14	F-31	1 year	1	B os	48 h	Maculopapular rash
15	F-21	4 years	1	B os	12 h	Maculopapular rash
16	F-29	6 years	2	PG im	10 h	Maculopapular rash
				B os	2 h	Maculopapular rash
17	F-46	5 years	1	Am im	1 h	Maculopapular rash
18	F-52	15 years	2	PG im	10 h	Angioedema
				Am os	6 h	Urticaria
19	F-22	3 months	2	Am os	12 h	Maculopapular rash
				Ax os	12 h	Maculopapular rash
20	F-34	10 years	1	B os	48 h	Maculopapular rash
21	M-17	1 month	2	Ax os	24 h	Maculopapular rash
				Ax os	6 h	Maculopapular rash
22	F-48	4 months	1	B os	48 h	Maculopapular rash
23	F-63	10 days	2	Am im	4 h	Maculopapular rash
				Pi im	8 h	Maculopapular rash
24	M-25	17 years	1	B os	8 h	Urticaria
25	F-63	2 years	2	Ax os	9 h	Urticaria, angioedema
				Ax os	9 h	Angioedema
26	M-17	5 months	1	B os	12 h	Erythema, pruritus
27	F-31	5 years	2	Am os	8 h	Maculopapular rash
				Am os	1 h	Maculopapular rash
28	F-31	1 year	1	Ax os	12 h	Maculopapular rash
29	F-58	5 months	1	Ax os	24 h	Maculopapular rash
30	M-32	5 months	2	Am os	4 h	Urticaria, syncope
				Ax os	3 h	Urticaria

Abbreviations: see footnote to Table 2.

In most cases (36 episodes, ie, 81.8%), the drug was administered orally; only in a few cases (7 episodes, ie, 15.9%) parenterally. In one case only (2.3%), the patient (n. 1) developed angioedema of the face after the mere application of penicillin powder.

Symptoms arose after a mean time of 15 ± 2.4 hours (mean $\pm$ std. error) following drug administration (Table 3).

The reported symptoms were mainly cutaneous: maculopapular rash in 28 episodes (63.6%), urticaria-angioedema in 13 (29.5%), and erythema-pruritus in 3 (6.8%). In addition to cutaneous symptoms, patient 1 presented also dyspnea and syncope, and patient 30 reported syncope in one of the two reported episodes.

The great majority of the investigated patients (29 out of 30 cases) had a positive patch test to the drug responsible for the reported adverse reactions: only patient 10, who reported an adverse reaction to cefalexin, showed a negative patch test with this drug (although positive to ampicillin, amoxicillin, bacampicillin, mezlocillin, ticarcillin, and cefuroxime).

A total of 252 tolerance challenge tests were performed, and their results are summarized in Table 2. According to patch positivity patterns and challenge tests, we were able to divide the investigated patients into three groups.

(1) *Group A*: Sixteen patients (53.3%; cases 2, 5, 7, 9, 11, 13, 14, 15, 19, 20, 21, 22, 25, 26, 27, 28) with patch test positivities to aminopenicillins only (amoxicillin, ampicillin, and bacampicillin). All other patch tests resulted invariably negative, and all natural and semisynthetic penicillins (others than aminopenicillins), monobactams, and carbapenems were tolerated.

In this group we found a cross-reactivity between aminopenicillins and aminocefalosporins in one case only (6.2%, patient n. 27), in which the tolerance oral challenge with cefalexin resulted positive, although the patch-test with this drug was negative. This finding was ascribed to antigenic cross-reactivity, due to the common α -NH₂ SC shared by aminopenicillins and aminocefalosporins. In this case,

however, the cross-reactivity was only partial because the oral challenge with cefalexin only resulted in positive reactions, while challenges with other aminocefalosporins were negative.

In six patients of this group (cases 5, 9, 20, 22, 25, 27), a provocative oral challenge with aminopenicillins was performed: in these patients, cutaneous symptoms closely resembling those obtained by history were observed within an interval ranging between 30 minutes and 48 hours. Symptoms were immediately treated with oral antihistamines and cortisone.

(2) *Group B*: Three patients (10.0%; cases 1, 18, 30), with positive patch tests to natural and semisynthetic penicillins only. These findings led us to suspect an allergy to the thiazolidine ring. Actually, both patch tests and the successive challenges with monobactams, carbapenems, and cephalosporins (where THIAZ is absent) were negative.

In patient 18 a positive tolerance challenge with penicillin G and ticarcillin was observed although patch-tests with these drugs were negative. The patient had a history of two adverse reactions to betalactams, one with penicillin and the other to ampicillin. She was positive to patch tests with ampicillin, amoxicillin, bacampicillin, and mezlocillin only. She underwent an intramuscular provocative challenge with mezlocillin, which resulted positive. This was predicted by the positive patch-test. In this particular case, the results of challenges were very helpful to indicate the presence of allergy to THIAZ.

(3) *Group C*: Eleven patients (36.7%; cases 3, 4, 6, 8, 10, 12, 16, 17, 23, 24, 29), in which patch tests did not follow any precise pattern of specificity. In these subjects, antigenity does not seem to be related to any clear haptenic determinant in the betalactam molecule, being rather patchy distributed and substantially unpredictable.

In this group we found the highest number of mis-matches between patch and challenge test results: 9 tolerance challenges (81.8%; patients n. 4, 8, 10, 16, 29) with betalactams previously

negative to patch tests (and non responsible for the reported adverse reactions) were unexpectedly positive.

It is our opinion that in 7 patients of this group (cases 4, 6, 10, 12, 16, 23, 29) the NAD may act as the prevalent responsible epitope, accounting for a frequent cross-reactivity between penicillins and cephalosporins (in 5 cases reactions to cefalexin were observed, in 3 cefuroxime was involved, in 1 cefatrizine, in 1 cefamandole, in 1 cefalotin). The remaining 4 patients could have been sensitized to a more complex structure resulting from a different folding of the molecule, instead of the traditional epitopes. An epitope common to all betalactams that could result variously masked/exposed in different patients could be also involved. In fact, the actual antigenicity of betalactams should be considered as resulting from both chemical structure and final folding to endogenous protein (after BLR opening).

Five provocative challenges with the drug responsible for the reported adverse reactions were carried out in group C subjects (patients n. 4, 6, 17, 23), all resulting in positive reactions.

Actually, all 13 provocative challenges performed in our patients were positive (6 in group A, 2 in group B, and 5 in group C), determining the onset of symptoms closely resembling those reported in history. It's interesting to notice that for these symptoms (mostly cutaneous) cortisone was necessary, as antihistamines alone (which are appropriate in immediate allergy) were not sufficient.

The identification of the above mentioned subgroups appears to be promising in order to recognize the ability of the allergic patients to tolerate other betalactam antibiotics. In fact (Table 2), we observed that a total of 12 out of 252 tolerance tests (4.8% of cases, in 23.3% of all investigated subjects) were positive in spite of the negative result of the corresponding patch test. In other words, in our patients a negative patch test had a fairly good specificity, with a very impressive predictive value (95.2%) in indicating the

ability to tolerate other betalactam administrations with no cross-reactivity.

In patients belonging to groups A and B, a mis-match between patch and tolerance tests was observed in just 3 out of 178 cases (1.7% of cases, 10.5% of subjects; predictive value of a negative patch test = 98.3%), while such a mis-match in group C patients occurred in as much as 12.2% of cases (45.5% of subjects; predictive value = 87.8%), with a significant statistical difference (chi-square = 12.6, d.f. = 1; $P < .01$ in case distribution; chi-square = 4.7, d.f. = 1; $P < .05$ in patient's distribution).

Even if we arbitrarily restrict the statistical analysis (see "Discussion") to the 20 patients who did have a maculopapular rash (which is considered more typical of cell-mediated allergic reactions), the results appear substantially unchanged. In fact, we couldn't observe any statistical difference in patient's distribution among groups (11 subjects in group A, none in group B, and 9 in group C). Further, a mis-match between patch and tolerance tests occurred in a total of 10 out 167 cases (6.0%, 30.0% of all subjects), with a predictive value of a negative patch test (94.0%) almost identical to the one previously observed in the overall population. Likewise, the mis-match prevalence in group A (0.9% of cases, 9.1% of patients; predictive value of a negative patch test: 99.1%) was significantly lower than in group C (15.5% of cases, 55.6% of patients; predictive value: 84.5%) (chi-square = 14.3, d.f. = 1; $P < .01$ in cases distribution; chi-square = 5.1; d.f. = 1; $P < .05$ in patient's distribution).

DISCUSSION

Our results clearly indicate that the systemic reactions to betalactams may have a cell-mediated pathogenesis.

In the interpretation of our data, we should however consider that betalactams (mainly penicillin) administration may result in different immunologic responses, which may be also independent of a strictly allergic clinical reaction. In particular, an aspecific immunologic response may develop in many

patients receiving penicillin (which has a high immunogen power) with no adverse reactions, as demonstrated by *in vitro* lymphocyte proliferation tests.²² In our opinion, the occurrence of such a simple "aspecific" reaction to betalactams should be excluded in our patients (all showing a positive patch test). In fact, to our knowledge, in the literature (and in our controls) no positive patch test has been observed in subjects who have previously tolerated penicillin administration. Further, the hypothesis of an aspecific reaction to betalactams should be definitely ruled out in our patients by the strict association observed among reported symptoms, positive patch tests and (in some cases) the positive result of provocative challenge tests with the responsible drug itself.

It is well-known that the ability to detect IgE decreases over time after an initial IgE-mediated allergic reaction,²⁴⁻²⁷ even though hypersensitivity may be elicited by re-exposure to the specific allergen. On this basis, the possibility that our results could have been colored by the admixture of a few patients who could have had an IgE-mediated reaction (having now no longer detectable IgE) might be somehow pertinent, although unlikely. In fact, we have to consider that in most cases (Table 3) the time elapsed since the first reported reaction was not enough to justify a normal IgE level. Furthermore, in the investigated subjects the presence of negative skin tests together with a clearly positive patch test does strongly support the occurrence of a cell-mediated reaction, which was further confirmed by the histologic results (skin biopsies).

To definitely rule out such a hypothesis, we restricted the statistical analysis to the patients who presented a maculopapular rash only (which is considered typical of cell-mediated reactions), excluding the subjects with urticaria-angioedema (in whom the hypothesis of a few cases with an IgE-mediated reaction might be less unlikely). Even in such a restricted group, our results were similar to those obtained in the overall investigated pop-

ulation. This fact constitutes a further indirect confirmation of the actual occurrence of a true cell-mediated allergic reaction in all our patients.

Unfortunately, the use of patch tests (able to detect the occurrence of a type IV, cell-mediated, hypersensitivity mechanism) is not widely recognized in drug allergy.^{28,29}

On the basis of the obtained results, however, we should consider patch tests as extremely safe (since no side effects were observed) and fairly specific. In our opinion, the diagnostic approach to patients with allergy to betalactams should include patch tests, in addition to skin (prick and intradermal) tests and RASTs (which detect a type I, IgE-mediated hypersensitivity only).

Patch tests should be carried out with a broad variety of molecules (such as PPL, MDM, penicillin G, ampicillin, amoxicillin, piperacillin, ticarcillin, imipenem, aztreonam, cefalexin, cefuroxime, ceftriaxone, and ceftibuten) in order to have a clear pattern of cross-reactivities and specificities for each patient.

A striking result obtained in our study is the high prevalence of patients with positive patch tests to aminopenicillins (29 out of 30 subjects). This fact suggests a major role of the α -NH₂ side-chain in determining the antigenicity of the molecule, confirming previous literature reports.^{12,14,30,31} The SC may act as a specific antigen, like in group A (allergy to α -NH₂ side chain), or it may potentiate other epitopes in the penicilloyl structure, like in groups B (allergy to THIAZ) and C (undetermined specificity).

Delayed (cell-mediated) allergy to aminopenicillins alone should be therefore considered as a definite and distinct nosologic entity when compared with other types of allergy to betalactams. It is characterized by some of the following typical findings:

- a maculopapular rash is the most typical symptom, arising a few hours after drug administration;
- cross-reactivity with aminocephalosporins is usually absent, or it is

limited to cefalexin, while other cephalosporins are usually tolerated;

- all natural and semisynthetic penicillins other than aminopenicillins are tolerated, as well as monobactams and carbapenems.

Tolerance challenge tests revealed a cross-reactivity among different betalactam molecules in 11 patients only (36.7%), mostly in group C (Table 2). A cross-reactivity between natural and semisynthetic penicillin was documented in 10 patients (33.3% of cases, all of them belonging to groups B or C). Cross-reactivity between penicillins and cephalosporins was found in 8 out of 30 patients only (26.6% of cases): one in group A and 7 in group C. In no case was it complete because at least a few cephalosporins (mainly cefitibuten) were always well tolerated (even in group C). Aminocephalosporins, and particularly cefalexin, were the most likely to show general cross-reactivity.

We found no positive tolerance test to monobactams (aztreonam) and/or carbapenems (imipenem), even when they were referred as the responsible drug. On this basis, it should be considered safe to administer carbapenems and monobactams to patients with previous adverse reactions to betalactams (unless these molecules are referred to as responsible drugs), as previously suggested also by literature reports concerning IgE-mediated allergy to monobactams³²⁻³⁴ and carbapenems.³⁵

Oral and intramuscular challenges appeared to be highly useful in confirming the results of negative patch tests, allowing the assesment of allergic patients to given diagnostic groups in order to select safe alternative betalactam molecules for each investigated subject.

A total of 252 tolerance challenge tests were attempted in 30 patients. If guided by an initial hypothesis on the epitope responsible for the allergy of any single patient and by patch tests' results, the tolerance challenge tests appeared to be reasonably safe in the greatest majority of cases. Only in 4.8% of cases (12 challenges), tolerance tests were positive in spite of the

negative result of the corresponding patch test, revealing the occurrence of cross-reactivity not discovered by patch tests.

Our results clearly demonstrated the reliability of a negative patch test in indicating the patient's ability to actually tolerate other betalactam administrations with no cross-reactivity, with a very impressive overall predicting value (95.2%). The predicting ability appeared particularly remarkable (98.3%) in subjects with allergy to SC of aminopenicillins (group A) or with allergy to THIAZ (group B). In the group where antigenicity was not clearly determined (group C), the occurrence of cross-reactivity seems to be less predictable by means of patch tests only. The careful use of tolerance challenge tests carried out on the basis of patients' history and patch-test results, however, made it possible to find alternative safe molecules to recommend for the future also for group C subjects.

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