

## CEPHALOSPORIN ALLERGY: CHARACTERIZATION OF UNIQUE AND CROSS-REACTING CEPHALOSPORIN ANTIGENS

A. ROMANO, D. QUARATINO, I. AIMONE-GASTIN\*, C. MAYORGA\*\*, G. PAPA,  
A. VENUTI, J.L. GUÉANT\* and M. BLANCA\*\*

*Department of Internal Medicine and Geriatrics, UCSC - Allergy Unit, C.I. Columbus, Rome, Italy;*

*\*Laboratory of Chemistry Proteins, Lyon, France; \*\*Research Unit for Allergic Diseases, Carlos Haya Hospital, Málaga, Spain*

Twelve subjects who had developed immediate allergic reactions after the administration of a cephalosporin were studied in order to identify the allergenic determinants involved in the induction of the reactions and to study the cross-reactivity with penicillins and with cephalosporins of different generations.

Skin tests and assays of IgE antibodies specific to determinants of benzyl penicillin (BP), amoxicillin (AX) and ampicillin (AMP) were carried out. In addition, skin tests and IgE-radioimmunoassays (RIA) were performed with the cephalosporins responsible. In some patients who presented skin-test positivity only to cephalosporins, cross-reactivity among these drugs was studied by skin testing with a panel of first-, second-, and third-generation cephalosporins.

Skin testing for immediate hypersensitivity demonstrated positive reactions to cephalosporins in 10 subjects, with IgE-RIA confirmation in 5. The other two patients presented IgE-RIA positivity with skin-test negativity.

Six out of the 12 subjects developed a positive response to cephalosporins and to one or more penicillin determinants, indicating cross-reactivity with penicillins. The other 6 developed a positive response only to cephalosporins. In this group, two patterns of reactivity were observed: one where the specificity seemed to be limited to the cephalosporin responsible and another where cross-reactivity among different cephalosporin structures existed.

Betalactams are the most important cause of adverse drug reactions mediated by an immunological mechanism (1, 2). The increasing use of new compounds such as second- and third-generation cephalosporins has created new specificities which may be responsible for sensitization and the ensuing allergic reactions (3). Of all betalactams, penicillins are the most frequently involved in such reactions. They are also the ones which have been most thoroughly studied (2, 4), because they are the most widespread (3) and the conjugates of their derivatives are stable (5, 6). The major antigenic determinant of benzylpenicillin is benzilpenicilloyl, which derives from nucleophilic bonding between the penicillin molecule and the amino group of proteins present

in both plasma and cell membranes (1, 5, 6). Cephalosporins also conjugate with proteins, forming the cephalosporoyl determinant, the equivalent of the penicilloyl determinant of penicillins (7). Less is known, though, about the immunological response to this structure (1), perhaps because of the greater fragmentation of this group (8). However, the side-chain structures usually survive and appear to be a major factor in the cross-reactivity between penicillins and cephalosporins, even though they may also be responsible for selective sensitization. Moreover, the production of hibridomae has shown that, in addition to determinants shared with penicillins, cephalosporins can generate unique determinants capable of inducing a specific immunological

*Key words: allergy, cephalosporins, cross-reactivities, immediate hypersensitivity, IgE, penicillins*

Mailing address: Dr. Antonino Romano  
Ambulatorio di Allergologia  
Complesso Integrato Columbus  
Via Pineta Sacchetti, 506  
00168 Roma, Italy  
Tel. 00 39 6 3503235 - Fax 00 39 6 3503653

0394-6320 (1997)

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response not cross-reacting with classic structures (9).

Thus, sensitivity to cephalosporins may occur because of cross-reactivity with betalactam ring structures, but selective IgE-mediated reactions to these compounds may also exist (10-13).

Most investigators have studied cross-reactivity with cephalosporins in penicillin-allergic subjects (8, 14-16), but there are few reports of cross-reactivity with penicillins in patients with a primary cephalosporin allergy (10-13). In the present study, a group of subjects who had developed an immediate allergic reaction to cephalosporins was assessed. A protocol for evaluating allergic reactions to betalactams was used. In addition to determinants generated by the culprit cephalosporin, classic determinants used for evaluating allergy to penicillins were employed. Results indicate that both selective and cross-reacting sensitization may occur.

## MATERIALS AND METHODS

### *Patient selection*

Patients were selected from a large outpatient population with histories of immediate reactions to cephalosporins and positive skin tests and/or IgE-radioimmunoassays (RIA) to these betalactam antibiotics.

### *Skin tests*

As previously reported (17, 18), these were carried out with the following reagents (the concentrations shown were the highest): benzylpenicilloyl polylysine (BPO-PLL) (Allergopharma Merck, Reinbeck, Germany) ( $5 \times 10^{-5}$  M), minor determinant mixture (MDM) (Allergopharma) ( $10^{-2}$  M), benzylpenicillin (PG) (Pharmacia, Milan, Italy) (10,000 IU/ml), amoxicillin (AX) (Smith Kline Beecham, Milan) (20 mg/ml) and ampicillin (AMP) (Amplital, Pharmacia) (20 mg/ml). Skin tests with culprit cephalosporins were also performed: cefotaxime (Zariviz, Hoechst Roussel, Milan, Italy), cefamandole (Mandokef, Lilly, Sesto Fiorentino, Italy), cephalixin (Keforal, Lilly), cefixime (Cefixoral, Menarini, Florence, Italy), ceftriaxone (Rocefin, Roche, Basel, Switzerland), ceftazidime (Glazidim, Glaxo, Verona, Italy), cefaclor (Panacef, Lilly), cefuroxime (Curoxim, Glaxo), cefmetazol (Cemetol, Pharmacia-Upjohn, Madrid, Spain). The maximum concentration used for the cephalosporin derivatives was 2 mg/ml. This concentration proved to be nonirritant in a negative control group of 30 subjects. In order to minimize the risks of systemic reactions, skin tests were started at lower concentrations (1/10,000 - 1/1,000 of maximum) in all patients whose

allergic reaction was strong or had occurred during the previous 6 months. Prick tests were used, but if they were negative at the maximum concentration, intradermal tests were performed. The criteria for defining a positive skin-test response were those previously reported (18).

### *Specific IgE antibodies*

Tests with BPO-PLL, ampicilloyl polylysine (AMP-PLL), and amoxicilloyl polylysine (AX-PLL) were carried out in the same way as reported elsewhere (19). A negative control group of subjects with no history of allergy to penicillin and skin-test negative to all the penicillin determinants mentioned above was used for determining the cut-off point. Values over the mean plus 2 SD of a negative control group were considered positive: 1.32 for BPO-PLL, 1.20 for AX-PLL and 1.12 for AMP-PLL. IgE antibodies to cephalosporins were detected by RIA performed as described previously (20). The results were expressed as the percentage of labelled anti-IgE adsorbed to the drug solid phase via IgE of the patient's serum (21). Percentages at least twice those obtained with control sera were considered positive. The specificity of IgE binding to the drug solid phase was established by inhibition in the presence of the soluble drug.

## RESULTS

A total of 12 subjects were investigated, 6 females and 6 males, with a mean age of 43 years (range 6-72 years). Anaphylaxis occurred in 7 cases and urticaria in 5. The cephalosporins most frequently involved in the induction of the reactions were cephalixin and ceftriaxone, which produced reactions on 3 occasions, followed by ceftazidime (2 occasions) and cefuroxime, cefixime, cefaclor, cefoxitine and cefmetazol (one occasion). In 10 patients the episode was the only allergic reaction experienced after the administration of a betalactam derivative, while in the other 2 subjects previous reactions had occurred. Case 5 had experienced three reactions, the first and second of which was urticaria after intramuscular ceftriaxone administration and the third an anaphylactic shock after ceftazidime. Case 7 had first developed urticaria with benzylpenicillin and subsequently urticaria-angioedema with cephalixin. In all cases, the time interval between drug administration and the appearance of the symptoms was less than 1 h. Table I presents the results of skin tests for BPO, MDM, AX and AMP, and RAST for BPO-PLL, AMP-

| Pt. | Skin tests |     |    |    |     | RASTs |      |     |
|-----|------------|-----|----|----|-----|-------|------|-----|
|     | BPO        | MDM | PG | AX | AMP | BPO   | AX   | AMP |
| 1   | +          | +   | +  | +  | +   | 8.0   | 1.3  | 1.1 |
| 2   | -          | -   | -  | -  | -   | 0.1   | 0.1  | 0.2 |
| 3   | -          | -   | -  | -  | -   | 0.1   | 0.1  | 0.3 |
| 4   | -          | -   | -  | -  | -   | 0.1   | 0.1  | 0.2 |
| 5   | -          | -   | -  | -  | -   | 1.0   | 0.1  | 0.4 |
| 6   | -          | -   | -  | -  | -   | 5.7   | 0.2  | 0.3 |
| 7   | +          | -   | -  | -  | -   | 3.9   | 0.3  | 0.5 |
| 8   | -          | -   | -  | -  | +   | 0.3   | 0.6  | 5.7 |
| 9   | +          | +   | -  | -  | -   | 3.5   | 0.1  | 0.2 |
| 10  | -          | -   | -  | -  | -   | 0.7   | 0.4  | 0.3 |
| 11  | -          | -   | -  | -  | -   | 0.9   | 0.7  | 0.5 |
| 12  | +          | +   | +  | -  | -   | 13.8  | 12.7 | 5.9 |

**TABLE I.** Results of skin tests and RASTs with penicillin determinants

| Pt. |             |      | Skin tests |  | RIA                     |          |              |
|-----|-------------|------|------------|--|-------------------------|----------|--------------|
|     |             |      |            |  | Patients/Controls Ratio | % Uptake | % Inhibition |
| 1   | Cephalexin  | P +  |            |  | 1.4                     | 5.0      | 0            |
| 2   | Ceftriaxone | ID + |            |  | 1.3                     | 10.5     | 0            |
| 3   | Ceftriaxone | ID + |            |  | 1.1                     | 8.9      | 0            |
| 4   | Ceftazidime | P +  |            |  | 4.4                     | 15.9     | 67           |
| 5   | Ceftriaxone | ID + |            |  | 8.7                     | 67.4     | 84           |
|     | Ceftazidime | P +  |            |  | 9.5                     | 34.1     | 82           |
| 6   | Cephalexin  | ID - |            |  | 2.8                     | 9.0      | 61           |
| 7   | Cephalexin  | ID + |            |  | 1.1                     | 3.9      | 0            |
| 8   | Cefaclor    | ID + |            |  | 2.6                     | 3.7      | 81           |
| 9   | Cefoxitine  | ID - |            |  | 5.0                     | 31.2     | 28           |
| 10  | Cefixime    | ID + |            |  | 1.0                     | 2.4      | 11.3         |
| 11  | Cefuroxime  | P +  |            |  | 2.6                     | 2.8      | 65           |
| 12  | Cefmetazol  | ID + |            |  | 9.6                     | 23.4     | 69           |

**TABLE II.** Results of skin tests and IgE-Radioimmunoassays (RIA) with cephalosporins.

*P* = prick test, *ID* = intradermal test

PLL and AX-PLL. Skin tests were positive with regard to one or more determinants in 5 cases.

Specific IgE antibodies were detected in 6 cases. Five of them showed positivity to BPO, while case 8 was RAST-positive only to AMP. It is interesting to note that the latter case was also skin-test positive only to AMP.

Table II shows the skin-test results for the cephalosporins studied. Ten cases turned out to be positive. In 6 patients the results were obtained by intradermal skin testing, and in cases 1, 4 and 11 by prick testing. Case 5 presented both prick-test positivity to ceftazidime and intradermal-test positivity to ceftriaxone.

IgE-RIA results are also presented in Table II. Seven cases were positive to the culprit cephalosporin. In case 5, specific IgE antibodies

for the two cephalosporins involved in the reactions were detected. Cases 6 and 9 were RIA-positive, but skin-test negative.

Six cases were also positive to penicillin determinants. All were positive to BPO except subject 8, who was skin-test positive to ampicillin and cefaclor and had serum specific IgE to both ampicillin and cefaclor, indicating that the antibody can recognize the side-chain structure shared by these two betalactams.

Table III displays skin-test results for different cephalosporins in 5 subjects of the group that was positive only to these compounds. It can be observed that in 3 cases there was a selective response to the cephalosporin responsible for the reaction. Two cases presented a skin-test response to two or more cephalosporins, including the culprit one.

| Pt. | CL | CM | CZ | CT | CU | CX |
|-----|----|----|----|----|----|----|
| 2   | -  | -  | -  | +  | -  | -  |
| 3   | -  | -  | -  | +  | -  | -  |
| 4   | -  | -  | +  | -  | -  | -  |
| 5   | -  | -  | +  | +  | +  | +  |
| 11  | -  | -  | -  | +  | +  | -  |

**TABLE III.** Results of skin tests with different cephalosporins

CL = Cephalotin  
 CM = Cefamandole  
 CZ = Ceftazidime  
 CT = Ceftriaxone  
 CU = Cefuroxime  
 CX = Cefotaxime

## DISCUSSION

Most of the studies carried out on betalactam allergies are based on responses to penicillins or unidentified betalactams (4). Furthermore, when cross-reactivity with cephalosporins has been studied the subjects selected were primarily chosen as allergic to penicillins, and cross-reactivity with cephalosporins was studied by selecting one or two of these compounds (8, 14-16). In the present study, the subjects were mainly selected on the basis of cephalosporin immediate hypersensitivity. Thus, this group was very suitable for establishing whether it was a question of primary sensitization to cephalosporins or cross-reactivity to the benzyl penicillin group.

In addition to penicillin determinants, we decided in a first approach to test with the cephalosporin involved in the reaction. However, in five cases we also performed skin tests with other cephalosporins in order to assess cross-reactivity among these compounds.

In this study 50% of the cases presented positive responses only to cephalosporins, while in the others positivity also appeared to one or more penicillin determinants. As Table III indicates, in the former group patients 2, 3 and 4 only reacted to the culprit cephalosporin, the drugs involved being ceftriaxone and ceftazidime. In cases 5 and 11, cross-reactivity with the other cephalosporins appeared, the responsible drugs being ceftazidime, ceftriaxone and cefuroxime. Although the sample

studied was very small, no positivity to first-generation cephalosporins appeared in this group. The third-generation cephalosporin ceftriaxone was the one most frequently involved in this cephalosporin-specific group, a fact which seems to be related to increased use of this compound with respect to the other cephalosporins.

It is important to note that the cephalosporin IgE-RIA proved to be useful in demonstrating immediate hypersensitivity to these drugs: it was negative in 5 skin-test-positive subjects, positive in 2 skin-test-negative ones, and confirmed skin-test positivity in the other 5. The question of whether subjects who reacted to cephalosporins did so because of cross-reactivity with betalactams or because of concomitant sensitivities is interesting, but difficult to answer in many instances. Although even after extensive research our group has not discovered any such concomitant sensitivities, some authors suggest that they may exist (22). The results of this study show that in cases who develop an immediate allergic reaction to a cephalosporin derivative, three main possibilities may occur. Subjects may present cross-reactivity with determinants of the penicillin group (either the penicilloyl group or the side-chain structure) or reactivity to determinants from the cephalosporin group, or they may react only to a specific cephalosporin determinant and thus do not show cross-reactivity with the other cephalosporins. Further studies are required in order to characterize this phenomenon more precisely.

## REFERENCES

1. Dewdney, J.M. 1977. Immunology of the antibiotics. In *Antigens 5*. Sela M. ed. New York: Academic Press, p. 73.
2. Weiss, M.E. and N.F. Adkinson. 1988. Immediate hypersensitivity reactions to penicillin and related antibiotics. *Clin. Allergy* 18: 515.
3. Blanca, M. 1995. Allergic reactions to penicillins. A changing world? *Allergy* 50: 777.
4. Blanca, M., J.M. Vega, J. García, A. Miranda, M.J. Carmona and C. Juárez. 1994. New aspects of allergic reactions to beta lactams: cross-reactions and unique specificities. *Clin. Exp. Allergy* 24: 407.
5. Batchelor, F.R., J.M. Dewney and D. Gazzard. 1965. Penicillin allergy: the formation of the penicilloyl determinant. *Nature* 206: 362.
6. Levine, B.B. and Z. Ovary. 1961. Studies of the mechanism of the formation of the penicillin antigen III. The N(D-(Benzyl penicilloyl) group as an antigenic determinant responsible for hypersensitivity to penicillin. *G. J. Exp. Med.* 114: 875.
7. Batchelor, F.R., J.M. Dewdney, R.D. Weston and A.W. Wheeler. 1966. The immunogenicity of cephalosporin derivatives and their cross-reaction with penicillin. *Immunology* 10: 21.
8. Assem, E.S.K. and M.R. Vickers. 1974. Test for penicillin allergy in man. II. The immunological cross-reaction between penicillins and cephalosporins. *Immunology* 27: 255.
9. Nagakura, N., S. Souma, T. Shimizu and Y. Yanagihara. 1991. Anti-cephalexin monoclonal antibodies and their cross-reactivities to cepheims and penems. *Int. Arch. Allergy Appl. Immunol.* 93: 126.
10. Igea, J.M., J. Fraj, I. Davila, M. Cuevas, J. Cuesta and M. Hinojosa. 1992. Allergy to cefazolin: study of *in vivo* cross reactivity with other betalactams. *Ann. Allergy* 68: 515.
11. Marcos Bravo, C., I. Luna Ortiz and R. Gonzales Vazquez. 1995. Hypersensitivity to cefuroxime with good tolerance to other betalactams. *Allergy* 50: 359.
12. Warrington, R.J. and S. McPhillips. 1996. Independent anaphylaxis to cefazolin without allergy to other betalactam antibiotics. *J. Allergy Clin. Immunol.* 98: 460.
13. Weber, E.A. 1996. Cefazolin specific side chain hypersensitivity. *J. Allergy Clin. Immunol.* 98: 849.
14. Blanca, M., J. Fernandez, A. Miranda, S. Terrados, M.J. Torres, J.M. Vega, M.J. Avila, E. Pérez, J.J. García and R. Suau. 1989. Cross-reactivity between penicillins and cephalosporins: clinical and immunological studies. *J. Allergy Clin. Immunol.* 83: 381.
15. Miranda, A., M. Blanca, J.M. Vega, F. Moreno, M.J. Carmona, J.J. García, E. Segurado, J.L. Justicia and C. Juárez. 1996. Cross-reactivity between a penicillin and a cephalosporin with the same side chain. *J. Allergy Clin. Immunol.* 98: 671.
16. Audicana, M., G. Bernalola, I. Urrutia, S. Echechipia, G. Gastaminza, D. Muñoz, D. Fernandez and L. Fernandez de Corres. 1994. Allergic reactions to betalactams: studies in a group of patients allergic to penicillin and evaluation of cross-reactivity with cephalosporins. *Allergy* 49: 108.
17. Blanca, M., J.M. Vega, J. García, M.J. Carmona, S. Terrados, A. Miranda, M.J. Avila and C. Juárez. 1990. Allergy to amoxicillin with good tolerance to other penicillins; study of the incidence in subjects allergic to betalactams. *Clin. Exp. Allergy* 20: 475.
18. Vega, J.M., M. Blanca, J.J. García, M.J. Carmona, A. Miranda, M. Pérez-Estrada, S. Fernández, J.M. Acebes and S. Terrados. 1994. Immediate allergic reactions to amoxicillin. *Allergy* 49: 317.
19. Blanca, M., C. Mayorga, E. Pérez, R. Suau, C. Juárez, J.M. Vega, M.J. Carmona, M. Pérez-Estrada and J. García. 1992. Determination of IgE antibodies to the benzyl penicilloyl determinant. A comparison between poly-L-lysine and human serum albumin as carriers. *J. Immunol. Methods* 153: 99.
20. Guéant, J.L., E. Mata, C. Masson, P. Gérard, D.A. Moneret-Vautrin, C. Mouton-Faivre and M.C. Laxenaire. 1995. Non-specific cross-reactivity of hydrophobic serum IgE to hydrophobic drugs. *Molec. Immun.* 32: 259.
21. Baldo, B.A. and M.M. Fischer. 1983. Anaphylaxis to muscle relaxant drugs. Cross-reactivity and molecular basis of binding of IgE antibodies detected by radioimmunoassay. *Molec. Immun.* 20: 1393.
22. Ong, R. and T. Sullivan. 1988. Detection and characterization of human IgE to cephalosporin determinants (Abstract). *J. Allergy Clin. Immunol.* 81: 222.