

# Delayed hypersensitivity to aminopenicillins is related to major histocompatibility complex genes

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**Background:** Although in some cases delayed hypersensitivity may be observed, beta-lactam antibiotics frequently induce immediate allergic IgE-mediated reactions with the specificity localized in the acyl-side chain structure. Generally, delayed immunologic reactions are related to sensitized T lymphocytes and major histocompatibility complex restricted.

**Objective:** To investigate the prevalence of HLA class I and II antigens in patients with delayed hypersensitivity to aminopenicillins in order to evaluate a relationship between major histocompatibility complex immune response genes and aminopenicillins hypersensitivity.

**Methods:** We assessed 24 patients with history of delayed hypersensitivity to aminopenicillins using (1) skin test with penicilloyl polylysine, minor determinant mixture, benzylpenicillin, amoxicillin, and ampicillin; (2) patch tests with benzylpenicillin, amoxicillin, and ampicillin; (3) RAST for penicilloyls G and V; and (4) oral challenges with amoxicillin, ampicillin, and penicillin V in 18/24 patients. All patients were typed by microlymphotoxicity standard test for HLA class I and II antigens. Statistical analysis by  $\chi^2$  test  $2 \times 2$  contingency tables, according to Svejgaard, were used for comparison between patients and random Italian population (522 subjects).

**Results:** In the patients group we found higher prevalence of HLA A2 (12/24 = 50%, RR = 6.76  $P < .001$ , EF = 0.425), DRw52 (20/24 = 83.3%, RR = 9.28,  $P < .001$ , EF = 0.74), and lower frequency of DR4 (3/24 = 12% ns).

**Conclusions:** These data suggest that the immune mechanisms involved in adverse reactions to aminopenicillins in vivo are related to genetic markers of immune response and confirms that the presentation of penicillin-hapten determinants to lymphocyte is major histocompatibility complex restricted.

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## INTRODUCTION

The role of HLA immune response genes in allergic diseases has been debated; indeed numerous studies have suggested an association between HLA genes and allergy.<sup>1</sup> In particular the possible role of HLA class II antigens in allergies to pollen allergens has been investigated and studies have yielded

conflicting results.<sup>2,3</sup> Since allergens are proteins with a complex structure and only some residues of the peptides could extend into the binding groove of HLA molecules, reports of positive association with HLA antigens are restricted to a few specific allergenic determinants.<sup>4</sup>

Although most allergic reactions to beta-lactam antibiotics are type I (IgE mediated), delayed (type IV) immunologic reactions to these drugs have also been reported as well as maculopapular eruptions.<sup>5</sup> Aminopenicillins (APs) show an amino group in the (D-) alpha position in the molecule (Fig 1), which has been demonstrated as the

cause of delayed hypersensitivity.<sup>6</sup> For this reason, this type of reaction may escape detection if a patch test is performed with benzyl-penicillin (BP) alone.<sup>6</sup> In vivo and in vitro studies of epidermal infiltrating T lymphocytes isolated from beta-lactam antibiotic-induced exanthems demonstrated the presence of a CD8+ subset with cytotoxic potential, usually antigen-specific and class II MHC-related.<sup>7</sup> Further, an aberrant expression of HLA-DR has been observed in keratinocytes isolated from drug-induced toxic epidermal necrolysis.<sup>8</sup> All these data support the hypothesis that HLA genes have a major role in delayed hypersensitivity to aminopenicillins.

The aim of this study was to investigate the prevalence of HLA class I and II antigens in patients with delayed hypersensitivity to aminopenicillins in order to evaluate the role of major histocompatibility complex (MHC) immune response genes in aminopenicillin hypersensitivity.

## MATERIALS AND METHODS

This study included 24 patients with delayed hypersensitivity (delayed intradermal and patch test positivity) to aminopenicillins. These 24 patients belonged to a group of 60 subjects who experienced maculopapular rashes during aminopenicillins therapy and accepted this specific research and diagnostic protocol.<sup>9</sup>

### *Immunoallergologic Tests Used for the Diagnosis*

*Skin tests* (prick and intradermal) were performed using the following: (a) PPL (Allergopen-Reinbeck, Germany); (b) MDM (Allergopen); (c) potassium penicillin G (PG) (Bristol Italiana-

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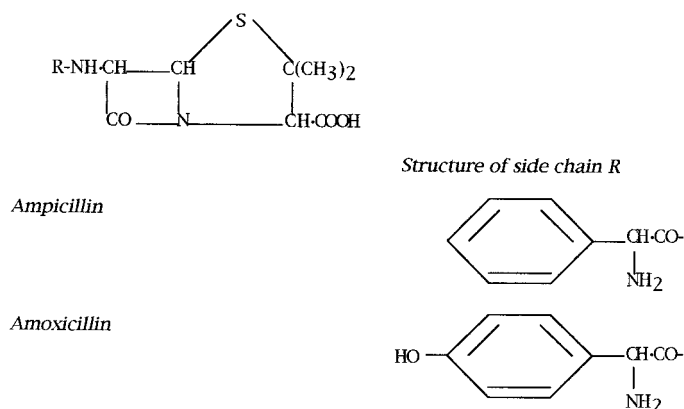


Figure 1. Aminopenicillins

Sermoneta, Italy) used at increasing concentrations ranging from 0.2 IU/mL to 2000 IU/mL (diluted in 0.9% NaCl); (d) ampicillin (AM) (Amplital, Farmitalia-Milan, Italy) and amoxicillin (AX) (Velamox, Zambelletti-Milan, Italy) diluted in 0.9% NaCl and used at a concentration of 1 mg/mL. All of the haptens were initially tested on the volar forearm skin using the prick technique. Reactions were considered positive when a wheal >3 mm in diameter was present 15 to 20 minutes later. When prick tests were negative, 0.02 mL of the hapten solution was injected intradermally within the volar forearm skin. Readings were made 20 minutes and 48 hours after injections. Results were defined as positive when wheals >5 mm were present. Positive controls for prick and intradermal tests were tested by histamine (at 10 and 1 mg/mL, respectively). Normal (0.9%) saline solution was used as a negative control.

**Patch tests** were carried out by (a) penicillin G (5000 IU/g in vaseline), (b) ampicillin (5% in vaseline), and (c) amoxicillin (5% in vaseline). The patches were applied to the interscapular area of the back and read after 48 and 72 hours. Results were defined according to the criteria of Wilkinson et al.<sup>10</sup>

**RAST** (radioallergosorbent test). In vitro assays were performed by the Phadebas RAST (Pharmacia-Uppsala, Sweden) for antigen-specific IgE to penicilloyls G and V.

**Oral challenge.** Eighteen of the 24 patients agreed to submit to oral challenges with penicillin V (Fenospin, Farmitalia), as well as with ampicillin (Amplital, Farmitalia) or amoxicillin (Velamox, Zambelletti), and only six patients refused their consent to the oral challenge. The patients were admitted to the day hospital for 24 hours. The aminopenicillins were initially administered at a dose of 5 mg. If no reaction occurred, the challenge was repeated with 50 mg and, eventually, with 500 mg. The penicillin V challenge was initially administered at a dose of 1,000 IU and repeated, when negative, at 10,000 IU and eventually with 1,000,000 IU. All repeated challenges were administered 1 week after the previous challenge.

**HLA classes I and II antigens** were typed by microlymphotoxicity standard test (Terasaki plates, Biotest,

Germany). The  $\chi^2$  analysis by  $2 \times 2$  contingency tables, according to Svejgaard,<sup>11</sup> were used for comparison between patients and a random Italian population (522 subjects). The strength of association between HLA-phenotypes and the disease was expressed as a relative risk (RR) and etiologic fraction (EF). Relative risk expresses the number of time risk of disease increased in individuals with marker present versus individuals with marker absent. Etiologic fraction represents the proportion of disease due to marker associated with the disease (Table 1).

## RESULTS

Table 2 shows clinical data, results of allergologic investigations and HLA typing, in each of the 24 patients (21 females and 3 males; age range, 13 to 61 years; and mean age,  $33.5 \pm 12.17$  years). Seven had experienced more than one episode of maculopapular eruption during aminopenicillins therapy. All patients were RAST negative.

In 10 of the 24 delayed hypersensitivity subjects, the maculopapular eruption was accompanied by angioedema. Seven subjects reported that the maculopapular rash had gradually assumed an urticarial aspect due to confluence of the original lesions. All 24 subjects claimed that their symptoms had appeared 24 hours or more after administration of aminopenicillins. The skin lesions persisted for several days after the antibiotic had been discontinued, in spite of corticosteroid

Table 1. Methods of Calculation of Relative Risk and Etiologic Fraction

| Basic data for calculation of risk estimate | Number of Individuals with Marker Present  |   | Marker Absent |
|---------------------------------------------|--------------------------------------------|---|---------------|
|                                             | Patients                                   | a | b             |
|                                             | Controls                                   | c | d             |
| Risk Estimate                               | Equation                                   |   |               |
| Relative risk                               | $\frac{a \times d}{b \times c}$            |   |               |
| Etiologic fraction                          | $\frac{RR - 1}{RR} \times \frac{a}{a + b}$ |   |               |

Table 2. History Data and Allergologic Investigation. Results of Cases Studied\*

| Sex | Age (yr) | Drug | Clinical Manifestations | Delayed intradermal positivity | Patch test positivity | Positive challenges | Dose responsive (mg or UI) | HLA typing                       |
|-----|----------|------|-------------------------|--------------------------------|-----------------------|---------------------|----------------------------|----------------------------------|
| F   | 23       | AM   | MP+SS                   | AM,AX                          | AM,AX                 | AM                  | 5                          | A1,B12<br>DR11(5),DRw52          |
| F   | 61       | AM   | MP                      | AM,AX                          | AM,AX                 | AM                  | 5                          | A2,A26, B blank<br>DR14,DRw52    |
| M   | 17       | AM   | MP+AE                   | AM,AX                          | AM,AX                 | AM                  | 50                         | A2,A29,B18<br>DR1,DR5,DRw52      |
| F   | 37       | AM   | MP+AE                   | AM,AX                          | AM,AX                 | AM                  | 50                         | A3,A9,B5<br>DR1,DR7,DRw52        |
| M   | 25       | BC   | MP                      | AM,AX                          | AM,AX                 | AM                  | 5                          | A1,B13<br>DR4,DRw13,DRw52-       |
| F   | 40       | BC   | MP                      | AM,AX                          | AM,AX                 | AM                  | 50                         | A2,B12<br>DR3,DR7,DRw52          |
| F   | 17       | BC   | MP+AE                   | AM,AX                          | AM,AX                 | AM                  | 5                          | A2,A9,B15<br>DR5,DR11,DRw52      |
| F   | 48       | AM   | MP+AE                   | AM,AX                          | AM,AX                 | np                  | np                         | A3,A26,B49<br>DR2,DRw52          |
| F   | 40       | AX   | MP+SS+fever<br>MP+SS    | AM,AX                          | AM,AX                 | AX                  | 50                         | A2,B13,B14<br>DR2,DR3,DRw52      |
| F   | 44       | AM   | MP                      | AM,AX                          | AM,AX                 | AM                  | 50                         | A2,A10,B18<br>DRw11,DRw52        |
| F   | 30       | AX   | MP                      | AM,AX                          | AM,AX                 | AX                  | 5                          | A1,A9,B57<br>DR2,DR11,DRw52      |
| F   | 35       | AX   | MP+AE                   | AM,AX                          | AM,AX                 | np                  | np                         | A26,Bw73<br>DR2,DRw11,DRw52      |
| F   | 40       | AX   | MP                      | AM,AX                          | AM,AX                 | AX                  | 50                         | A26,B13<br>DR3,DR7,DRw52-53      |
| F   | 17       | AM   | MP+SS                   | AM,AX                          | AM,AX                 | AM                  | 5                          | A2,A23,B49<br>DR3,DR5,DRw52      |
| F   | 37       | AM   | MP                      | AM,AX                          | AM,AX                 | np                  | np                         | A10,A31,B17,B18<br>DR3,DR5,DRw52 |
| F   | 33       | AM   | MP+AE+SS                | AM,AX                          | AM,AX                 | AM                  | 5                          | A2,B21,B35<br>DR1,DR7,DRw53      |
| F   | 52       | BC   | MP                      | AM,AX                          | AM,AX                 | AM                  | 5                          | A2,A26,B8<br>DR3,DRw52           |
| F   | 21       | AX   | MP                      | AM,AX                          | AM,AX                 | AX                  | 5                          | A2,A26,B14<br>DR1,DR7,DRw53      |
| F   | 28       | BC   | MP+AE<br>MP             | PG,AM,AX                       | PG,AM,AX              | np                  | np                         | A36,A23,B38<br>DR3,DR4,DRw52     |
| M   | 13       | AM   | MP                      | AM,AX                          | AM,AX                 | AM                  | 5                          | A9,B8<br>DR2,DRw52               |
| F   | 30       | AM   | MP+AE<br>MP+AE          | PG,AM,AX                       | PG,AM,AX              | np                  | np                         | A2,Aw36,B12<br>DR2,DR3,DRw52-53  |
| F   | 31       | AX   | MP                      | AM,AX                          | AM,AX                 | AX                  | 5                          | A9,Bw73<br>DR1,DR9,DRw53         |
| F   | 29       | AM   | MP+SS                   | AM,AX                          | AM,AX                 | np                  | np                         | A9,Bw63<br>DR4,DR7,DRw53         |
| F   | 47       | AM   | MP                      | PG,AM,AX                       | PG,AM,AX              | PG<br>AM            | 1000<br>5                  | A2,B18<br>DR3,DR5,DRw52          |

\* AM = ampicillin; AX = amoxicillin; BC = bacampicillin; PG = penicillin G; AE = angioedema; MP = maculopapular eruption; SS = skin scaling; and np = not performed.

therapy. In some cases, the skin underwent a desquamative phase prior to healing.

All subjects presented delayed intradermal and patch test positivity indicating delayed hypersensitivity to am-

picillin and amoxicillin; three were also positive for penicillin G. For the 18 patients who agreed to take the oral

Table 3. Prevalence of HLA A2 and DR w52 on the Population Studied

| HLA   | Patients % | Controls % | RR*  | EF*   | P     |
|-------|------------|------------|------|-------|-------|
| A2    | 50         | 12.8       | 6.76 | 0.425 | <.001 |
| DRw52 | 83.3       | 35         | 9.28 | 0.74  | <.001 |

\* RR = relative risk and EF = etiologic fraction.

challenge (six patients refused the challenge), delayed hypersensitivity was confirmed in all 18 patients.

In the patients group, the HLA class I antigen A2 was present in 50% of cases (12/24, RR = 6.76  $P$  < .001, EF = 0.425) and HLA class II antigens DRw52 in 83.3% (20/24 RR = 9.28,  $P$  < .001, EF = 0.74) (Table 3) whereas DR4 was present in 12% of subjects (3/24). The common haplotypes A2/DRw52 and A2/DR3/Drw52 were also found (16.6% and 20.8%, respectively).

## DISCUSSION

Although urticaria, Quincke's edema, and Arthus reactions are frequent during benzylpenicillin treatment, late onset reactions have been observed mainly after aminopenicillin use. Further, late onset maculopapular eruptions are a fairly common type of adverse delayed reaction to penicillins in general and ampicillin in particular.<sup>12</sup> In some cases they resemble viral exantems and are frequent with coexisting Epstein Barr virus or cytomegalovirus infection or administration of allopurinol.<sup>13</sup> Vega et al<sup>14</sup> proposed that the pathogenesis of maculopapular eruptions is an immune reaction mediated by amoxicillin and ampicillin-sensitized T lymphocytes. In two of four cases described, histopathologic studies showed a skin mononuclear infiltration without signs of leucocytoclastic vasculitis. Further, Terrados et al<sup>15</sup> in 6 of 29 subjects with "nonimmediate" reactions to beta-lactams found, by skin biopsies, a lymphocyte and mononuclear infiltrate but no immunoglobulins or complement, suggesting a type IV cell-mediated reaction. These data were also confirmed by other authors.<sup>16</sup> Particularly, Hertl et al<sup>17</sup> supported this hypothesis by demonstrating in two beta-lactam-allergic patients

that the epidermal T-cell subset in skin biopsies were predominantly CD8+ T-lymphocytes.<sup>7</sup> Since these T lymphocytes, isolated and expanded in vitro, proliferated in response to penicillin-pulsed, autologous antigen-presenting cells but not to allogenic antigen-presenting cells, they concluded that those clones were antigen specific and MHC restricted. Recent data by Padovan et al<sup>17</sup> also support the view that the antigenic epitopes recognized by T cells isolated from penicillin-allergic individuals are true hapten determinants and that their presentation is MHC restricted.

Many factors appear to be very important in investigating HLA gene susceptibility to diseases: the clinical phenotype must be precisely defined in relationship to a specific antigen. Indeed allergologic reports of association with HLA antigens are restricted only to specific allergens<sup>18,19</sup>: Ansari et al<sup>4</sup> found that immune responsiveness to a highly purified *Lolium perenne* pollen component was significantly associated with DR3 and B8 HLA antigens. Similarly, isocyanate-induced asthma is HLA class II allele restricted.<sup>19</sup> Further, in allergic patients T cells from peripheral blood and bronchoalveolar lavage are of the IL4-producing TH2 subset, whereas in non-allergic asthma they are of  $\gamma$ -interferon-producing TH1 subset.<sup>19</sup> Conversely, an association between atopy and the DR4 and DR7 HLA class II alleles suggest that these alleles are more involved in a general susceptibility to atopy rather than in a specific sensitivity to particular allergens.<sup>20</sup> All these studies increased the knowledge of the role of the immune system in the pathophysiology of allergic disease and reveal a new possibility for immunotherapeutic approaches using synthetic peptides, recombinant allergens,

degraded allergenic extracts, or modified allergens that selectively tolerize Th2-type or activate Th0-type or Th1-type responses to allergens.<sup>21</sup>

In our study all 24 patients were strictly selected: they showed maculopapular rashes during aminopenicillin therapy 24 hours or more after aminopenicillin treatment, and the skin lesions persisted for several days after the aminopenicillin had been discontinued and corticosteroid therapy had been started. The positive oral challenges in 18/24 patient, who agreed to submit to the oral test, supported the specificity of the skin tests (patch test and delayed intradermal test) for the diagnosis of delayed hypersensitivity to aminopenicillins, as we demonstrated in a previous study.<sup>9</sup> In contrast, all 16 subjects with negative allergologic tests were negative to the oral challenge performed with the culprit aminopenicillin. The preponderance of women (21/24 patients observed) with maculopapular eruptions and evidence of delayed type hypersensitivity to aminopenicillins reflects the female preponderance of the initial sample of 195 patients reporting adverse cutaneous reactions to aminopenicillins, in which 147 were females.<sup>9</sup> The significance of this female preponderance is unknown.

HLA typing showed a significant increase of A2 (50%) and DRw52 antigens (83.3%). The more frequent common haplotype was A2/DRw52 and A2/DR3/Drw52 (16.6% and 20.8%, respectively). DRw52a has been described previously on 100% of patients with primary sclerosing cholangitis.<sup>22</sup> Since this antigen is present in all patients studied the authors proposed that the determinant of sclerosing cholangitis susceptibility is on position 26 (tyrosine), 38 (leucine), 57 (valine), or 60 (serine), all of which are unique to DRw52a. Further, at present no associations between common haplotype A2/DRw52 and A2/DR3/Drw52 have been demonstrated in other autoimmune diseases. All these data suggest that HLA class I and II haplotypes are specifically implicated in the regulation of the immune response to beta-

lactam antibiotics. Investigation by molecular biologic methods such as polymerase chain reaction-restriction fragment-length polymorphism (PCR-RFLP) will probably identify a more specific allelic variation associated with either resistance or susceptibility to delayed reaction to beta-lactams, consistent with well-known associations between autoimmune disorders and allelic variations in the HLA region.<sup>19</sup> Since the acyl-side chain has a role in the specificity of delayed immunologic reactions to beta-lactam,<sup>9,23</sup> we speculated that the amino-group in the (D-) alpha position or a more extensive determinant site consisting of the penicilloyl structure together with the side chain,<sup>24</sup> or a neo-antigen resulting from polymerization of aminopenicillin molecule,<sup>25</sup> may be recognized by HLA molecules. Such is the case in IgE-mediated reactions to penicillins where MHC-restricted T cells recognize both side chain and core structure of penicillins.<sup>17</sup> This interaction explains (1) the specificity of skin reaction, (2) the particular subset of skin infiltrating lymphocytes, (3) the late response after oral challenge, and (4) the association with Epstein Barr virus infection, which makes B lymphocytes act as antigen-presenting cells.

In conclusion, genes of immune response in the HLA region, as demonstrated for other specific allergens,<sup>18</sup> are related to adverse delayed reactions in aminopenicillin-exposed subjects. Accordingly, antigenic modulation by penicillin analogues could be useful to desensitize or to prevent reactions to beta-lactam antibiotics.

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