

## Original article

# Distinct delayed T-cell response to $\beta$ -methasone and penicillin-G in the same patient

**Background:** Multiple drug allergy syndrome is a clinical condition characterized by reactions against more than one different class of, both pharmacologically and structurally, unrelated drugs. Scanty data are available to date about a multiple drug delayed hypersensitivity syndrome. Our aim was to report the case of a delayed reaction to both  $\beta$ -methasone ( $\beta$ -MT) and penicillin-G (pen-G) occurring in the same patient, and analyse  $\beta$ -MT- and pen-G-specific T-cell Lines (TCLs) with regard to their specificity, phenotype and cytokine profile.

**Methods:** We generated two drug-specific TCLs from biopsies at the site of positive intradermal reactions, and analysed their immunophenotype, T-cell receptor V $\beta$  (TCR-V $\beta$ ) domains expression and cytokine profile.

**Results:** We demonstrated the specificity of the T cells isolated from positive intradermal test reactions to pen-G and  $\beta$ -MT through the strict dose-dependent proliferation in response to drug-pulsed autologous antigen presenting cells. Fluorescence activated cell sorter (FACS) analysis revealed a predominance of CD4+ cells in the inflammatory cell infiltrate of intradermal test with  $\beta$ -MT, while a predominance of CD8+ T cells in the site of delayed reaction to pen-G was found. The drug specific CD4+ and CD8+ T cells were heterogeneous, with regard to TCR-V $\beta$  usage. CD8+ pen-G-TCL displayed a preferential T helper 2 (Th2) profile, while a substantially heterogeneous pattern of cytokine production characterized specific  $\beta$ -MT TCL.

**Conclusion:** The study describes the coexistence in the same patient of a delayed hypersensitivity to both penicillin G and  $\beta$ -MT, driven, respectively, by pen-G-specific Th2-skewed CD8+ and  $\beta$ -MT specific Th0 CD4+ T cells. This case further support the existence of a multiple drug allergy syndrome also for delayed hypersensitivity.

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Drugs can induce several immune adverse reactions, classified, in the case of  $\beta$ -Lactams, on the basis of the time interval between drug administration and the onset of symptoms, as immediate (within 1 h), accelerated (between 1 and 72 h) and delayed (after 72 h) (1–3). The allergic responses to drugs comprise both immunoglobulin E (IgE)-mediated (immediate allergy) and T-cell mediated (delayed allergy) reactions (4). The diagnosis of these reactions is based on *in vivo* (skin prick test, intradermal, epicutaneous tests and provocation test) and *in vitro* tests (detection of drug-specific IgE and lymphocyte transformation test) (3, 5). There is *in vitro* evidence that T-cell mediated immune responses play an important, although still unclear, role in both immediate and delayed types of adverse drug reactions (6, 7). Recently, the generation of T-cell lines (TCL) and/or T-cell clones has provided more information about the characteristic and the molecular mechanisms involved in drug recognition by the specific T cells (6, 8–11).

Penicillin and related antibiotics with the  $\beta$ -Lactam structure represent drugs that most frequently induce immunologically mediated adverse drug reactions (1, 3, 12–14). The cutaneous reactions observed in penicillin-G (pen-G)-induced hypersensitivity consist of different clinical manifestations, ranging from urticaria and/or anaphylaxis to morbilliform and scarlatiniform eruptions, erythema multiforme, Steven–Johnson syndrome and toxic epidermal necrolysis (4, 11, 15, 16). The former types of reactions (immediate) are mainly IgE-mediated, whereas the latter forms of eruptions feature a predominant lymphocytic infiltrate, thus suggesting a T-cell involvement.

On the other hand, systemically administered corticosteroids appear to be an infrequent cause of immunologically mediated reactions (17–21). Among steroid-induced allergic reactions, the most frequent is the delayed hypersensitivity because of topical sensitization, with an incidence of 2.9–4.1% (22). Cross-reactions between corticosteroids are common events. Taking advantage

of cross allergic patterns between structurally related molecules, Dooms-Grossens et al. suggest that corticosteroids can be subdivided into four different groups (21–23):

- Class A (hydrocortisone type without substitution on the corticosteroid D-ring, C<sub>20</sub> or C<sub>21</sub>-chain, but including short chain esters such as acetate esters and the C<sub>21</sub>-thioester, tixocortol pivalate)
- Class B (triamcinolone acetonide type with a C<sub>16</sub>, C<sub>17</sub>, cis-ketal or -diol structure)
- Class C (betamethasone type with a C<sub>16</sub>-methyl substitution, non-esterified at C<sub>17</sub>, C<sub>21</sub>)
- Class D (hydrocortisone- and clobetasone-17-butyrate type corticosteroids with a long-chain ester at C<sub>17</sub> and/or C<sub>21</sub>).

Corticosteroid contact dermatitis is difficult to establish, as the anti-inflammatory and immunosuppressive properties of the tested substance may suppress skin reactions in patch testing (17). Furthermore, corticosteroids are used in anti-inflammatory treatment, and therefore are rarely considered as the potential source of an adverse reaction.

In this study, we report the immunological evaluations in a patient where a delayed reactivity to both pen-G and  $\beta$ -methasone ( $\beta$ -MT) driven by distinct T-cell populations coexisted.

## Methods

### Case report

A 41-year-old nonatopic man complained of pruritus, generalized urticaria and facial angioedema 2 days after oral administration of  $\beta$ -MT sodium phosphate. Two months later, he suffered a similar episode within 1 day from re-exposure to  $\beta$ -MT that persisted for several days. The patient was referred to our allergy clinic for aetiological diagnosis. After obtaining the patient's written consent, an allergy study was carried out. The patient had no personal or family history of allergy and he was not taking any concomitant therapy at the time of his adverse reaction. However, sensitization to common allergens and foods was ruled out on the basis of negative immediate hypersensitivity test. Immunoglobulin E levels were 45 kU/l and blood eosinophil count was 35 cell/ $\mu$ l.

The diagnostic procedures consisted of skin prick test and intracutaneous test with  $\beta$ -MT and methylprednisolone acetate as a control. Prick test used 0.1 and 1 mg/ml, intracutaneous tests were carried out with serial dilution from 0.001 to 1 mg/ml. No immediate reaction was observed. However, 48 h after the test, itching and erythematous patches developed at the injection sites of  $\beta$ -MT, while no reaction was observed at the injection sites of methylprednisolone acetate.

As it is widely known that corticosteroids can be subdivided into four different groups of structurally related substances (21–23), conventional patch tests with 2% hydrocortisone acetate (group A), 1% budesonide (group B), 10%  $\beta$ -MT (group C) and 1% clobetasol propionate (group D) were also performed. The substances were diluted in petrolatum, as described (22). Patch tests were applied under occlusion with Finn Chamber (Alpharma, Norgesplaster, Norway) on the upper back, and readings were carried out 2 and 4 days after

application. Slight reaction was observed for both budesonide and  $\beta$ -MT after 96 h, but not for the other corticosteroids.

Finally, the patient tolerated increasing doses of a group A steroid (methylprednisolone acetate, 1, 1 and 2 mg added every 30 min), reaching therapeutic dose, without complications.

Furthermore, the patient's historic data reported that a maculopapular eruption occurred about 36 h after aminopenicillin treatment, about 8 years before. Assays (UniCAP specific IgE; Pharmacia & Upjohn, Uppsala, Sweden) for specific IgE to penicilloyl G, penicilloyl V, ampicilloyl, amoxicilloyl and cefaclor were negative ( $<0.35$  kU/l). Hence, we also carried out a skin test with  $6 \times 10^{-5}$  mM/l, benzylpenicilloyl polylysine (BPO) (Allergopen, Reinbek, Germany),  $2 \times 10^{-2}$  mM/l minor determinant mixture containing benzylpenicillin and benzylpenicilloate (Allergopen), pen-G, ampicillin and amoxicillin, and patch tests with the last three drugs (3). A strong delayed hypersensitivity to pen-G and a slight reaction to amoxicillin were therefore diagnosed on the basis of both patch tests and delayed intradermal positivity.

### Generation of T-cell Lines

Five millimetres punch biopsy specimens at the site of the positive delayed reactions at intracutaneous test with  $\beta$ -MT and pen-G were obtained to isolate drug-specific TCL from skin infiltrating lymphocytes (SIL). To this end, the skin biopsy was washed with phosphate-buffered saline (PBS) and then placed in culture in antibiotic-free RPMI 1640 medium supplemented with 2 mM L-glutamine (Life Technologies, Chagrin Falls, OH) (complete RPMI), 10% heat-inactivated foetal calf serum (FCS) (HyClone, Logan, UT), and 60 U/ml rIL-2 (Boehringer Mannheim, Mannheim, Germany) for 2 weeks, with fresh medium and interleukin-2 (IL-2) replaced every 3–4 days.

The TCL obtained were assayed for antigen (Ag)-specificity after extensive washing to remove IL-2. Different stimulation procedures were chosen: (i) direct peripheral blood mononuclear cells (PBMC) and TCL stimulation with 'soluble Ag' at different doses of  $\beta$ -MT (from 1 to 100  $\mu$ M) or pen-G (from 0.5 to 3 mg/ml) concentrations and (ii) PBMC and TCL stimulation with the same number of drug-modified autologous PBMC, named Ag-pulsed antigen presenting cells (APC). Modification of APC was achieved by incubation of APC either with 1, 10 and 100  $\mu$ M  $\beta$ -MT, or 0.5, 1 and 3 mg/ml pen-G for 4 h. Then APC were washed twice with complete antibiotic-free RPMI 1640 medium and irradiated (6000 rad). Antigen-pulsed stimulatory cells were added to  $1 \times 10^6$  cell/well of the corresponding PBMC or TCL. After 48 h, cells were resuspended, and a 200  $\mu$ l aliquot was transferred into 96-well U-bottom microtiter plates. Proliferation of these cells was determined by overnight incubation with 0.5  $\mu$ Ci/ml [<sup>3</sup>H]thymidine (Amersham, Little Chalfont, UK). Cells were then harvested onto fibre-coated 96-well plates (Packard Instruments, Groningen, The Netherlands) and incorporated radioactivity was measured on a scintillation liquid-free  $\beta$ -counter (Topcount; Packard Instruments).

Peripheral mononuclear cells were isolated from 20 ml heparinized blood via density gradient centrifugation (Ficoll-Hypaque). After two wash cycles the peripheral mononuclear cells were incubated both with soluble Ag as described above, or with Ag-pulsed APCs.

### Immunophenotyping of TCLs and analysis of V $\beta$ domains of expressed TCR- $\beta$ chains

In this study the following monoclonal antibodies (MoAbs) were used: fluorescein isothiocyanate (FITC)-conjugated anti-CD7, CD25, CD27, CD30, CD45R0, CD60, CD69, CD103, CD124 and

cutaneous lymphocyte antigen (CLA); R-phycoerythrin (PE)-conjugated anti-CD26, CD28, CD38, CD45RA, and CD49d (VLA4), CD70 and  $\beta 7$ ; FITC, PE, or peridinin chlorophyll protein (PCP)-conjugated anti-CD3, CD4, or CD8 (Becton Dickinson Immunocytometry system, Mountain View, CA). Three-colour flow cytometry analysis was performed as previously described (24). Briefly,  $1 \times 10^5$  TCL suspension was mixed with 10  $\mu$ l of each MoAb and incubated in the dark for 30 min at 4°C. After 30 min, cells were washed twice in cold PBS and then resuspended in 300  $\mu$ l PBS containing foetal bovine serum and NaN<sub>3</sub> for immediate flow cytometry analysis. An irrelevant mouse IgG1 MoAb (Becton Dickinson) was used as a negative control.

The TCLs were also studied for TCR-V $\beta$  domain expression using a panel of 21 V $\beta$  mAbs: V $\beta$ 1 (BL37.2), V $\beta$ 2 (MPB2D5), V $\beta$ 3 (CH92), V $\beta$ 5.1 (IMMU157), V $\beta$ 5.2 (36213), V $\beta$ 5.3 (3D11), V $\beta$ 7 (ZOE), V $\beta$ 8.1/8.2 (56C5.2), V $\beta$ 9 (FIN9), V $\beta$ 11 (C21), V $\beta$ 12 (VER2.32.1), V $\beta$ 13.1 (IMMU222), V $\beta$ 13.6 (JU-74), V $\beta$ 14 (CASI.1.3), V $\beta$ 16 (TAMAYA 1.2), V $\beta$ 17 (E17.5F3), V $\beta$ 18 (BA62), V $\beta$ 20 (ELL 1.4), V $\beta$ 21.3 (IG125), V $\beta$ 22 (IMMU 546) and V $\beta$ 23 (AF 23). The TCR-V $\beta$  antibodies were from Immunotech (Marseilles Cedex, France). Data acquisition and analysis of the TCR-V $\beta$  immunofluorescence stainings were performed in triple staining using 10  $\mu$ l unconjugated TCR-V $\beta$  mAbs, followed by incubation with 4  $\mu$ l PE-conjugated goat anti-mouse immunoglobulin (Dako, Glostrup, Denmark) and subsequently 4  $\mu$ l normal mouse serum (Sigma-Aldrich, Milan, Italy) to block free Ag-binding sites of the second-step reagent, after which CD4-FITC and CD8-PCP were added. To define single V $\beta$  expression, the mean normal V $\beta$  value was used. Quantitative analysis for three-colour flow cytometry was carried out using a FAC-Scan instrument (Becton Dickinson). At least 10 000 events were acquired in list mode, and all the data were analysed by the CellQuest software (Becton Dickinson). Live lymphomonocytes were electronically gated by forward and right angle scatters.

### Measurements of cytokines

Supernatants from the drug-specific TCL and PBMC were collected after 48 h cultures ( $10^6$  cells/ml in 24 wells) in complete RPMI supplemented with 5% human serum in the presence of 10 ng/ml phorbol myristate acetate (PMA) and 1  $\mu$ g/ml ionomycin (Sigma),

in order to provide a maximal stimulus, filtered, and then stored at  $-80^\circ\text{C}$ . Interferon- $\gamma$  (IFN- $\gamma$ ), CD30s, macrophage derived chemokine (MDC), thymus- and activation-regulated chemokine (TARC), IL-4, IL-5, IL-10, IL-12, IL-13, and IL-18 content was measured with commercially available sandwich ELISA kits (Quantikine; R&D Systems, Minneapolis, MN), following the manufacturer's instructions and the results were interpolated from the standard reference curve provided with each kit. The same cytokines were also measured in the serum of the patient.

### Results

T cells isolated from positive intradermal test reactions to pen-G and  $\beta$ -MT are drug specific

We took advantage of the coexistence of a delayed hypersensitivity to both  $\beta$ -MT and pen-G to perform two punch biopsies at the site of delayed reaction on the forearms, in order to isolate drug-specific TCL. We tested the TCLs obtained for drug specificity, both by using drug-pulsed autologous APC and adding the Ag directly to the cell cultures.

Stimulation of pen-G-skin-derived TCL with pen-G-pulsed APC resulted in a strong dose-dependent proliferation from 0.5 mg to 3 mg/ml (Fig. 1A). When we studied the  $\beta$ -MT-skin-derived TCL, the strongest proliferative response was achieved after incubation with 1  $\mu$ M  $\beta$ -MT-pulsed APC (Fig. 1B). To evaluate whether preactivated T cells reacted to drugs presented by nonclassical APC, TCLs were stimulated by adding the Ag directly to the cell cultures in the absence of any APC. Neither  $\beta$ -MT- nor pen-G-skin-derived TCL were stimulated by the continuous presence of either soluble  $\beta$ -MT or pen-G, showing that a classical Ag processing was required for presentation of both drugs.

In a similar way, we also tested the response of PBMC to Ag-pulsed APC. We found a strict dose-dependent

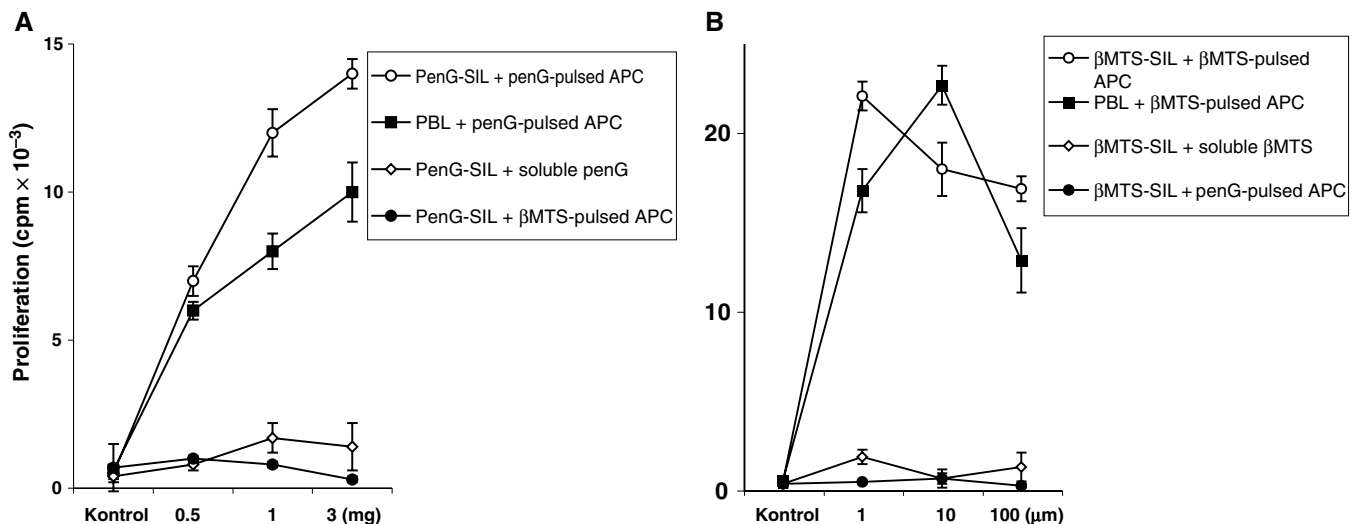


Figure 1. Dose-dependent proliferative response of skin infiltrating lymphocytes and PBMC to penicillin-G and  $\beta$ -methasone.

proliferation to pen-G-pulsed APC, while a preferential response at 10  $\mu$ M  $\beta$ -MT-pulsed APC was achieved (Fig. 1).

$\beta$ -Methasone-skin-derived TCL did not respond to pen-G-pulsed APC and, similarly, pen-G-skin-derived TCL did not respond to  $\beta$ -MT-pulsed APC.

Peripheral blood mononuclear cells from three nonallergic and noncontact sensitive donors did not respond to either Ag stimulation.

#### Flow cytometry analysis

The complete immunophenotype of T-cell derived drug-specific TCL are shown in Table 1. Comparing the two TCL, a distinct phenotype was found.  $\beta$ -Methasone-specific TCL were prevalently CD4+/ $\alpha\beta$ + T cells, whereas about 95% of penicillin-specific TCL were CD8+/ $\alpha\beta$ + T cells. Both TCL expressed the CD45R0 isoform. Penicillin-G-specific CD8+ T cells expressed a higher level of CD25, CD30, CD69, CD95 and CD103 when compared with  $\beta$ -MT-specific TCL. Surprisingly the  $\beta$ -MT-specific TCL expressed low levels of CD28 and very scant levels of CD27 (both the molecules were expressed by about the 95% of CD4+ T cells in the peripheral blood).

The distinct phenotype of pen-G-specific TCL and  $\beta$ -MT-specific TCL could also be reflected in a different use of TCR-V $\beta$  chain. To analyse this aspect we stained the pen-G- or  $\beta$ -MT-specific TCLs with TCR-V $\beta$ -specific antibody (Table 1). Both TCLs did not reveal a preferential use of a particular TCR-V $\beta$  chain. TCLs were also stained for TCR-V $\beta$  at different time points of the culture and also showed no up-regulation of a particular TCR-V $\beta$  chain (data not shown).

#### Cytokine pattern released by penicillin- and $\beta$ -MT-specific TCL

Since we found in the same patient a delayed reactivity to two nonrelated drugs driven by CD4 and CD8 T cells, respectively, we sought to investigate the cytokine pattern of these different reactive cells. The cytokine pattern of the drug-specific TCL generated from our patient was assayed before and after 48-h stimulation with PMA/Ionomycin. The value of IFN- $\gamma$ , CD30s, MDC, TARC, IL-4, IL-5, IL-10, IL-12, IL-13, and IL-18 are presented in Table 2. As shown in Table 2, we found a rather heterogeneous picture of released cytokines, which did not allow a strict classification into one defined T helper (Th) subtype. In both CD4 and CD8 T-cell clones, PMA/Ionomycin tended to increase the secreted levels of IFN- $\gamma$ , IL-4 and IL-13, and to decrease the levels of MDC, IFN-gamma-induced protein of 10 KD (IP-10) and TARC. Interestingly, IL-5 was constitutively overexpressed in pen-G TCL. Higher levels of IL-5, IL-13, CD30s, TARC, MDC and IL-10 could be observed in pen-G-specific TCL when compared with  $\beta$ -MT TCL irrespective of stimulation with PMA/Ionomycin, suggesting the presence of a preferential Th2-skewed profile in pen-G-specific TCL.

Table 1. Immunophenotype and TCR-V $\beta$  analysis of skin-derived TCLs

|                     | $\beta$ -MT reactive (%)                     | Pen-G reactive (%)                     |
|---------------------|----------------------------------------------|----------------------------------------|
| CD4+                | 92                                           | 5                                      |
| CD8+                | 8                                            | 95                                     |
| TCR $\alpha\beta$   | 100                                          | 100                                    |
|                     | Within CD3+ CD4+<br>$\beta$ -MT reactive (%) | Within CD3+ CD8+<br>Pen-G reactive (%) |
| Mature T cell       |                                              |                                        |
| CD7+                | 52                                           | 71                                     |
| CD27+               | 3                                            | 25                                     |
| CD28+               | 33                                           | 41                                     |
| CD45R0+             | 100                                          | 100                                    |
| Activation          |                                              |                                        |
| CD25+               | 39                                           | 73                                     |
| CD26+               | 18                                           | 20                                     |
| CD30+               | 5                                            | 17.5                                   |
| CD38+               | 27                                           | 45                                     |
| CD60+               | 9                                            | 7                                      |
| CD69+               | 16                                           | 27                                     |
| CD70+               | 53                                           | 54                                     |
| CD95+               | 23                                           | 40                                     |
| Adhesion            |                                              |                                        |
| CD103+              | 4                                            | 21                                     |
| CLA+                | 3                                            | 2                                      |
| $\alpha 4\beta 1$ + | 50                                           | 48                                     |
| $\alpha 4\beta 7$ + | 18                                           | 23                                     |
| IL-4 receptor       |                                              |                                        |
| CD124+              | < 0.1                                        | 7                                      |
|                     | CD4+<br>$\beta$ -MT reactive (%)             | CD8+<br>pen-G reactive (%)             |
| TCR-V $\beta$ 1     | 1.05                                         | 2.6                                    |
| TCR-V $\beta$ 2     | 8                                            | 2.7                                    |
| TCR-V $\beta$ 3     | 1.5                                          | 2.93                                   |
| TCR-V $\beta$ 5.1   | 5.06                                         | 2.51                                   |
| TCR-V $\beta$ 5.2   | <0.1                                         | 1.34                                   |
| TCR-V $\beta$ 5.3   | 1.35                                         | 0.37                                   |
| TCR-V $\beta$ 7     | 4.88                                         | 2.38                                   |
| TCR-V $\beta$ 8     | 5.52                                         | 2.21                                   |
| TCR-V $\beta$ 9     | 2.52                                         | 2.21                                   |
| TCR-V $\beta$ 11    | 1.42                                         | 1.64                                   |
| TCR-V $\beta$ 12    | 2.69                                         | 5.45                                   |
| TCR-V $\beta$ 13.1  | 6.23                                         | 2.69                                   |
| TCR-V $\beta$ 13.6  | 0.86                                         | 4.48                                   |
| TCR-V $\beta$ 14    | 2.76                                         | 0.7                                    |
| TCR-V $\beta$ 16    | 1.24                                         | 0.16                                   |
| TCR-V $\beta$ 17    | 3.74                                         | 1.59                                   |
| TCR-V $\beta$ 18    | 3.06                                         | 2.45                                   |
| TCR-V $\beta$ 20    | 2.74                                         | 1.54                                   |
| TCR-V $\beta$ 21    | 2.5                                          | 2.39                                   |
| TCR-V $\beta$ 22    | 3.99                                         | 2.21                                   |
| TCR-V $\beta$ 23    | 1.79                                         | 0.74                                   |

## Discussion

We report a case of delayed reaction to both  $\beta$ -MT and pen-G occurring in the same patient. We isolated drug-specific TCL from biopsies at the site of the positive intradermal test reactions to  $\beta$ -MT and pen-G and analysed them with regard to their specificity, phenotype and cytokine profile. The specificity was demonstrated by

Table 2. Cytokine profile of drug specific T-cell Lines

|                       | $\beta$ -MT TCL |                | Pen-G TCL       |                |
|-----------------------|-----------------|----------------|-----------------|----------------|
|                       | Before stimulus | After stimulus | Before stimulus | After stimulus |
| IFN- $\gamma$ (pg/ml) | 2               | 124            | 33              | 239            |
| CD30s (U/ml)          | 3               | 1.7            | 54              | 32             |
| MDC (pg/ml)           | 1859            | 1044           | 5076            | 2357           |
| IP-10 (pg/ml)         | 61              | 52             | 275             | 48             |
| TARC (pg/ml)          | 184.5           | 116            | 1535            | 563.6          |
| IL-4 (pg/ml)          | 0.7             | 41             | 15              | 38             |
| IL-5 (pg/ml)          | 16              | 23             | 855             | 901            |
| IL-10 (pg/ml)         | 40              | 13             | 60              | 127            |
| IL-12 (pg/ml)         | 15              | 12             | 12              | 15             |
| IL-13 (pg/ml)         | 7               | 135            | 2353            | 2735           |
| IL-18 (pg/ml)         | 6               | 2              | 17              | 17             |

the strong dose-dependent proliferation obtained in response to drug-pulsed autologous APC (Fig. 1). FACS analysis revealed a predominance of CD4<sup>+</sup> cells in the inflammatory cell infiltrate of intradermal test with  $\beta$ -MT, whereas a predominance of CD8<sup>+</sup> T cells in the site of delayed reaction to pen-G was found (Table 1). The preferential isolation of distinct TCL in the same patient strongly suggests that the delayed reaction to different drugs in our patient was mediated by distinct T-cell population. The drug specific CD4<sup>+</sup> and CD8<sup>+</sup> T cells were heterogeneous, with regard to TCR-V $\beta$  usage. Accordingly, although some drug-specific reactions can be oligoclonal (25), most drug specific TCL generated from the peripheral blood bear heterogeneous TCR (12, 26, 27).

It is widely known that drugs can be processed and presented in different ways by the immune system (28). Our data suggest that the patient described herein was able to present pen-G or  $\beta$ -MT in the context of major histocompatibility complex class I and class II, respectively. It has been suggested that different types of clinical manifestations are driven by different T-cell subsets. Indeed, the presence of CD8 Th1 like T cells correlated to bullous exanthemas, whereas the predominance of a CD4<sup>+</sup> Th2 like population was associated with urticarial exanthemas in pen-G allergic patients (4, 29). Hari et al. also investigated the T-cell activation and drug specificity in different forms of drug-induced exanthemas from 22 patients. They found that a predominant CD8(+) T-cell

activation gave more severe skin symptoms or liver involvement. In contrast, maculopapular reactions were observed in the presence of a predominant activation of CD4<sup>+</sup> cells (15).

In agreement with these reports, our patient complained of a more severe, CD8-driven reaction after pen-G intake (maculopapular rash followed by loss of consciousness), when compared with  $\beta$ -MT-induced, CD4-mediated generalized urticaria and facial angioedema.

Conflicting results have been reported with regard to the pattern of cytokines produced by  $\beta$ -Lactam specific TCL (12, 30–33). Pen-G specific T-cell clones were found to produce high amounts of IFN- $\gamma$ , showing a prevalent Th1 like pattern (12), while in other studies TCL derived from peripheral blood of patients with hypersensitivity to pen-G were predominantly CD4<sup>+</sup> cells with a Th2 profile of cytokine production (4). Brugnolo et al. have convincingly demonstrated a Th2 skewed cytokine profile in a panel of pen-G specific T cells clones (31). In contrast, a heterogeneous cytokine pattern was found by other authors (12, 32).

In our hands, pen-G TCL displayed a preferential Th2 profile, as demonstrated by the prominent release of IL-5, IL-13, TARC and MDC. By contrast, a substantially heterogeneous pattern of cytokine production was characteristic of specific  $\beta$ -MT TCL (Table 2).

Another topic of our study was the presence of a cross-reaction between  $\beta$ -MT and budesonide in the absence of any cross-reaction to hydrocortisone acetate, clobetasol propionate or methylprednisolone acetate. These results are in agreement with the expected pattern of cross-reactions between corticosteroids. In particular, budesonide (group B) has been reported to cross-react with corticosteroids belonging to C (like  $\beta$ -MT) and D groups (22, 34). In fact, our patients tolerated without complications the increasing doses of methylprednisolone acetate, a member of the group A corticosteroids.

Contrasting data are available about the possibility that several individuals more likely than others might develop immediate adverse drug reactions (35–39). The case we presented of a patient reactive both to  $\beta$ -MT and pen-G further support the existence of a multiple drug allergy syndrome also for delayed hypersensitivity (40).

## References

1. BLANCA M, TORRES MJ, GARCIA JJ, ROMANO A, MAYORGA C, DE RAMON E, VEGA JM, MIRANDA A, JUAREZ C. Natural evolution of skin test sensitivity in patients allergic to beta-lactam antibiotics. *J Allergy Clin Immunol* 1999;**103**:918.
2. ROMANO A, TORRES MJ, QUARATINO D, DI FONSO M, PERRONE MR, VIOLA M, VENUTI A, BLANCA M. Diagnostic evaluation of delayed hypersensitivity to systematically administered drugs. *Allergy* 1999;**54**(Suppl 58):23.
3. ROMANO A, QUARATINO D, DI FONSO M, PAPA G, VENUTI A, GASBARRINI G. A diagnostic protocol for evaluating nonimmediate reactions to aminopenicillins. *J Allergy Clin Immunol* 1999;**103**:1186.
4. HERTL M, MERK HF. Lymphocyte activation in cutaneous drug reactions. *J Invest Dermatol* 1995;**105**:95S.

5. NYFELER B, PICHLER WJ. The lymphocyte transformation test for the diagnosis of drug allergy: sensitivity and specificity. *Clin Exp Allergy* 1997;**27**:175.
6. LUQUE I, LEYVA L, JOSE TORRES M, ROSAL M, MAYORGA C, SEGURA JM, BLANCA M, JUAREZ C. Invitro T-cell responses to beta-lactam drugs in immediate and nonimmediate allergic reactions. *Allergy* 2001;**56**:611.
7. VON GREYERZ S, BURKHART C, PICHLER WJ. Molecular basis of drug recognition by specific T-cell receptors. *Int Arch Allergy Immunol* 1999;**119**:173.
8. PADOVAN E, MAURI-HELLWEG D, PICHLER WJ, WELTZIEN HU. T cell recognition of penicillin G: structural features determining antigenic specificity. *Eur J Immunol* 1996;**26**:42.
9. PADOVAN E, VON GREYERZ S, PICHLER WJ, WELTZIEN HU. Antigen-dependent and -independent IFN-gamma modulation by penicillins. *J Immunol* 1999;**162**:1171.
10. PICHLER WJ, SCHNYDER B, ZANNI MP, HARI Y, VON GREYERZ S. Role of T cells in drug allergies. *Allergy* 1998;**53**:225.
11. GONZALEZ FJ, LEYVA L, POSADAS S, LUQUE I, BLANCA M, SANTAMARIA L, JUAREZ C. Participation of T lymphocytes in cutaneous allergic reactions to drugs. *Clin Exp Allergy* 1998;**28**(Suppl 4):3.
12. BRANDER C, MAURI-HELLWEG D, BETTENS F, ROLLI H, GOLDMAN M, PICHLER WJ. Heterogeneous T cell responses to beta-lactam-modified self-structures are observed in penicillin-allergic individuals. *J Immunol* 1995;**155**:2670.
13. HERTL M, GEISEL J, BOECKER C, MERK H F. Selective generation of CD8+ T-cell clones from the peripheral blood of patients with cutaneous reactions to beta-lactam antibiotics. *Br J Dermatol* 1993;**128**:619.
14. TORRES MJ, MAYORGA C, GARCIA JJ, ROMANO A, JUAREZ C, BLANCA M. New aspects in betalactam recognition. *Clin Exp Allergy* 1998;**28**(Suppl 4):25.
15. HARI Y, FRUTIG-SCHNYDER K, HURNI M, YAWALKAR N, ZANNI MP, SCHNYDER B, KAPPELER A, VON GREYERZ S, BRAATHEN LR, PICHLER WJ. T cell involvement in cutaneous drug eruptions. *Clin Exp Allergy* 2001;**31**:1398.
16. ROMANO A, DI FONSO M, PAPA G, PIETRANTONIO F, FEDERICO F, FABRIZI G, VENUTI A. Evaluation of adverse cutaneous reactions to aminopenicillins with emphasis on those manifested by maculopapular rashes. *Allergy* 1995;**50**:113.
17. DOOMS-GOOSSENS AE, DEGREEF HJ, MARIEN KJ, COOPMAN SA. Contact allergy to corticosteroids: a frequently missed diagnosis? *J Am Acad Dermatol* 1989;**21**:538.
18. REINHOLD K, SCHNEIDER L, HUNZELMANN N, KRIEG T, SCHARFFETTER-KOCHANKE K. Delayed-type allergy to systemic corticosteroids. *Allergy* 2000;**55**:1095.
19. WHITMORE SE. Delayed systemic allergic reactions to corticosteroids. *Contact Dermatitis* 1995;**32**:193.
20. VIDAL C, TOME S, FERNANDEZ-REDONDO V, TATO F. Systemic allergic reaction to corticosteroids. *Contact Dermatitis* 1994;**31**:273.
21. COOPMAN S, DEGREEF H, DOOMS-GOOSSENS A. Identification of cross-reaction patterns in allergic contact dermatitis from topical corticosteroids. *Br J Dermatol* 1989;**121**:27.
22. DOOMS-GOOSSENS A, MORREN M. Results of routine patch testing with corticosteroid series in 2073 patients. *Contact Dermatitis* 1992;**26**:182.
23. MORREN MA, DOOMS-GOOSSENS A. Contact allergy to corticosteroids. Diagnosis and management. *Clin Rev Allergy Immunol* 1996;**14**:199.
24. SCALA E, RUSSO G, CADONI S, NARDUCCI MG, GIRARDELLI CR, DE PITA O, PUDDU P. Skewed expression of activation, differentiation and homing-related antigens in circulating cells from patients with cutaneous T cell lymphoma associated with CD7- T helper lymphocytes expansion. *J Invest Dermatol* 1999;**113**:622.
25. MAURI-HELLWEG D, ZANNI M, FREI E, BETTENS F, BRANDER C, MAURI D, PADOVAN E, WELTZIEN HU, PICHLER WJ. Cross-reactivity of T cell lines and clones to beta-lactam antibiotics. *J Immunol* 1996;**157**:1071.
26. YAWALKAR N, HARI Y, FRUTIG K, EGLI F, WENDLAND T, BRAATHEN LR, PICHLER WJ. T cells isolated from positive epicutaneous test reactions to amoxicillin and ceftriaxone are drug specific and cytotoxic. *J Invest Dermatol* 2000;**115**:647.
27. VON GREYERZ S, ZANNI MP, FRUTIG K, SCHNYDER B, BURKHART C, PICHLER WJ. Interaction of sulfonamide derivatives with the TCR of sulfamethoxazole-specific human alpha beta+ T cell clones. *J Immunol* 1999;**162**:595.
28. BASKETTER D, DOOMS-GOOSSENS A, KARLBERG AT, LEPOITTEVIN JP. The chemistry of contact allergy: why is a molecule allergenic? *Contact Dermatitis* 1995;**32**:65.
29. HERTL M, BOHLEN H, JUGERT F, BOECKER C, KNAUP R, MERK HF. Predominance of epidermal CD8+ T lymphocytes in bullous cutaneous reactions caused by beta-lactam antibiotics. *J Invest Dermatol* 1993;**101**:794.
30. YAWALKAR N, EGLI F, HARI Y, NIEVERGELT H, BRAATHEN LR, PICHLER WJ. Infiltration of cytotoxic T cells in drug-induced cutaneous eruptions. *Clin Exp Allergy* 2000;**30**:847.
31. BRUGNOLO F, ANNUNZIATO F, SAMPOGNARO S, CAMPI P, MANFREDI M, MATUCCI A, BLANCA M, ROMAGNANI S, MAGGI E, PARRONCHI P. Highly Th2-skewed cytokine profile of beta-lactam-specific T cells from nonatopic subjects with adverse drug reactions. *J Immunol* 1999;**163**:1053.
32. ZANNI MP, VON GREYERZ S, SCHNYDER B, MAURI-HELLWEG D, BRANDER C, KALBERMATTEN C, PICHLER WJ. T cell reactions in patients showing adverse immune reactions to drugs. *Inflamm Res* 1996;**45**(Suppl 2):S79.
33. PICHLER WJ, ZANNI M, VON GREYERZ S, SCHNYDER B, MAURI-HELLWEG D, WENDLAND T. High IL-5 production by human drug-specific T cell clones. *Int Arch Allergy Immunol* 1997;**113**:177.
34. OH-I T. Contact dermatitis due to topical steroids with conceivable cross reactions between topical steroid preparations. *J Dermatol* 1996;**23**:200.
35. KHOURY L, WARRINGTON R. The multiple drug allergy syndrome: a matched-control retrospective study in patients allergic to penicillin. *J Allergy Clin Immunol* 1996;**98**:462.
36. PHAM NH, BALDO BA, PUY RM. Studies on the mechanism of multiple drug allergies. Structural basis of drug recognition. *J Immunoassay Immunochem* 2001;**22**:47.
37. SCALA E, GIANI M, PIRROTTA L, GUERRA EC, DE PITA O, PUDDU P. Multiple drug allergy syndrome: severe anaphylactic reaction due to topical rifamycin SV in a patient with hypersensitivity to ciprofloxacin. *Int J Dermatol* 2001;**40**:603.
38. NETTIS E, COLANARDI MC, PAOLA RD, FERRANNINI A, TURSI A. Tolerance test in patients with multiple drug allergy syndrome. *Immunopharmacol Immunotoxicol* 2001;**23**:617.
39. ASERO R. Multiple drug allergy syndrome: a distinct clinical entity. *Curr Allergy Rep* 2001;**1**:18.
40. SCALA E, GIANI M, PIRROTTA L, GUERRA EC, GIRARDELLI CR, DE PITA O, PUDDU P. Simultaneous [correction of Spontaneous] allergy to ampicillin and local anesthetics. *Allergy* 2001;**56**:454.