

## Characterization of primary recall *in vitro* lymphocyte responses to bacampicillin in allergic subjects

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### Summary

**Background** Antigen-specific cell lines or clones are often used as models of drug-specific allergy. However, cloning procedures are time consuming, and the repeated antigen stimulation cycles as well as the addition of various growth enhancers may affect the *in vivo* relevance of these systems.

**Objective** Using bacampicillin-allergic subjects, we wanted to investigate the applicability of primary recall *in vitro* lymphocyte responses to characterize type I and type IV allergy. The sensitivity and specificity of LTT (Lymphocyte transformation test), when used as an *in vitro* diagnostic tool, were also assessed.

**Methods** A total of 39 patients with symptoms of type I (rhinitis) or type IV (allergic contact dermatitis, ACD) allergy following occupational exposure to bacampicillin, were included. Ten individuals without penicillin allergy or occupational exposure to bacampicillin served as controls. All subjects were LTT tested. Four patients with rhinitis and two patients with ACD were available for studying the immunophenotype and the TCR-V $\beta$  repertoire of bacampicillin induced lymphoblasts as well as the cytokine profiles and expression of the activation markers CD23 and CD134 in primary PBMC cultures.

**Results** LTT was positive in 87% and at least one of the skin tests was positive in 85% of the patients with allergic symptoms. 69% of the patients with type I allergies were patch test-positive. Results from LTT and skin test correlated in 87% of the cases. The combined sensitivity of LTT and skin tests was 92%. The specificity of LTT was 90% in healthy controls. Bacampicillin induced lymphoblasts were mainly CD4+ in both ACD and rhinitis patients. The TCR-V $\beta$  profiles of the predominant CD4+ lymphoblasts were heterogeneous with individual skewing towards V $\beta$ 2, V $\beta$ 3, V $\beta$ 5.1 and/or V $\beta$ 14. An increased expression of IFN $\gamma$  was detected in bacampicillin treated PBMC cultures from the ACD but not from rhinitis patients. IL-5 was detected in bacampicillin exposed PBMC cultures from all patients but not from healthy controls. This Th2 environment could also be verified by CD23 and CD134 expression.

**Conclusion** LTT and skin tests are equally sensitive in identifying bacampicillin allergic subjects. When the two tests are combined, the sensitivity increases. The patch test is useful not only for detection of type IV but also for the identification of type I allergies. When using primary PBMC cultures, IFN $\gamma$  is the most suitable cytokine to discriminate between type I and type IV allergy. IL-5 can possibly be used as a general marker for bacampicillin induced allergy. Thus, primary cell cultures may be considered as an alternative to T-cell lines or clones for the study of drug induced allergy.

**Keywords:** activation markers, allergic contact dermatitis, bacampicillin, cytokines, flow cytometry, human lymphocytes, lymphocyte transformation test, rhinitis, skin test, T-cell receptor V $\beta$

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## Introduction

Drug allergy is still a serious problem to society and further studies dealing with critical mechanisms behind these reactions is a current demand [1]. We routinely use the LTT, as a complement to skin test, in the diagnosis of occupationally related drug allergies, induced after exposure in pharmaceutical plants [2,3]. Bacampicillin has been most frequently tested drug. Allergic symptoms due to bacampicillin includes type IV-(allergic contact dermatitis, ACD), and type I-reactions (rhinitis and conjunctivitis). Penicillins are known to induce both type I and type IV reactions after occupational exposure through inhalation or skin contact [2] or when given therapeutically [4,5]. Beta-lactams are extensively studied drug-sensitizers [6–8], although the cytokine pattern [9] and the TCR-V $\beta$  repertoire [10] of penicillin-specific lymphocytes have not been conclusively documented. The majority of such studies have been performed using penicillin-specific cell lines or clones. However, the effects of repeated *in vitro* exposure to the particular drug, usually in combination with mitogens and recombinant cytokines for establishing these drug-specific cell lines or clones, are largely unknown. Early studies of the TCR construct have revealed that the CDR3 region is the contact site for recognition of antigens [11,12]. The CDR3 region is formed by both the V $\alpha$  and the V $\beta$  chain of the TCR. At present the TCR-V $\beta$  genes are grouped into about 26 and the V $\alpha$  genes into about 32 families [13,14] although monoclonal antibodies to V $\beta$  are more readily accessible than those to the V $\alpha$  structures. The aim of this study was to investigate the applicability of primary PBMC cultures derived from patients with occupational allergy to bacampicillin, to characterize allergic responses. Two subjects with type IV reactions and four subjects with type I reactions were tested for *in vitro* cytokine production over time, analysis of immunophenotype, the T-cell activation marker CD134 (OX40) [15], the B-cell activation marker CD23, and the expression of 18 different TCR-V $\beta$  chains on bacampicillin-induced lymphoblasts. The sensitivity of LTT and skin test in the diagnosis of bacampicillin-induced allergy was calculated using clinical history as a reference. Healthy controls without penicillin allergy were tested for cytokines and with LTT in order to calculate the specificity of LTT.

## Materials and methods

### Patients

Between 1990 and 1998, 39 penicillin workers, repeatedly exposed to bacampicillin dust in a pharmaceutical plant, were diagnosed at the occupational health services (Astra-Zeneca, Södertälje, Sweden) as allergic to bacampicillin based on their clinical history, Table 1. A total of 16 patients reported type I-, 19 patients type IV-, and four patients both type I and type IV symptoms. Blood samples from all patients were taken for LTT, followed by skin tests with bacampicillin. All tests were performed within 6 months of the clinical reaction. Four patients with rhinitis and two patients with ACD, all showing positive skin tests and LTT to bacampicillin, were willing to give additional blood samples. These patients were further tested for cytokine profiles and flow cytometry analysis of immunophenotype and TCR-V $\beta$  repertoire of bacampicillin-induced lymphoblasts. Ten healthy persons without penicillin allergy or occupational exposure to penicillins, were used as controls in LTT and three of them were also analysed for *in vitro* cytokine production.

### Patch test and prick test

Patch tests were performed on all patients. Patients with a history of type I allergy, and a negative patch test, were also prick tested. These skin tests were performed as previously described [2]. Bacampicillin was dissolved in water and used at the following concentrations: 1, 3, 6 and 10%.

### Lymphocyte transformation test (LTT)

The LTT was performed according to the MELISA® (memory lymphocyte immuno-stimulation assay) procedure, as described earlier [16].

Briefly, venous blood was defibrinated by shaking and mononuclear cells were isolated on Ficoll® gradients (Pharmacia, Uppsala, Sweden). Adherent cells were removed by plastic adherence and nonadherent cells diluted to  $1 \times 10^6$ /mL in RPMI medium with 10% AB+ and cultivated for 5 days in 1-mL cultures using 48-well plates (Costar, USA). Bacampicillin was tested at

**Table 1** Patient's clinical history

| Occupation          | Type I* | Type IV† | Type I and IV |
|---------------------|---------|----------|---------------|
| Process operator    | 10      | 12       |               |
| Tablet manufacturer | 2       | 2        | 1             |
| Cleaner             | 1       | 1        | 1             |
| Packer              |         | 1        |               |
| Laboratory engineer | 1       | 2        | 1             |
| Craftsman           | 1       | 1        | 1             |
| Foreman             | 1       |          |               |

\*Rhinitis/conjunctivitis/rhinoconjunctivitis were observed in all cases except one process operator who suffered from urticaria. No other skin reactions were reported in this patient group. †ACD/rash on skin exposed to bacampicillin, predominantly hands and face. No symptoms from mucosal membranes were reported in this patient group.

6.25–200 µg/mL. PPD (Purified Protein Derivative, tuberculin, Statens Seruminstitut, Denmark) was used at a concentration of 1 µg/mL as a positive control for memory-cell proliferation since the majority of the adult Swedish population has been immunized with bacille Calmette Guérin. SEB, Staphylococcus enterotoxin B (Sigma, USA), 10 ng/mL, was used as a positive control for specific stimulation of TCR-Vβ 3, –12, –14 and –17 in the flow cytometry analyses. To measure the cell proliferation, 600 µL of cell suspension from each culture was subsequently transferred to a new cultivation plate with 3 µCi <sup>3</sup>H-thymidine (Amersham, UK), specific activity 12.7 GBq/mg, in each well. After 5 hours the cell cultures were harvested and the <sup>3</sup>H thymidine incorporation, expressed as c.p.m., was measured by liquid scintillation in a beta counter (Wallac, Finland). Cytospin preparations were made from all the cell cultures on the last day of cultivation and stained with the May-Grünwald/Giemsa analogue Diff-Quik® (Baxter, Germany). The number of lymphoblasts was assessed by light microscopy. Stimulation indices (SI) (= c.p.m. in antigen treated culture/c.p.m. in culture without antigen) > 3 were considered as positive if confirmed by the presence of lymphoblasts.

#### Flow cytometry

The following monoclonal antibodies were used: FITC-conjugated: Vβ5.2 + 5.3, Vβ5.3, Vβ5.1, Vβ6.7, Vβ8, Vβ13.1, 13.3 (Endogen, USA), Vβ1, Vβ2, Vβ3, Vβ7, Vβ11, Vβ12, Vβ16, Vβ17, Vβ20, Vβ21.3, Vβ22, Panγδ (Immunotech, France), CD19, CD20, CD4 (Becton Dickinson). PE-conjugated: CD4, CD8, CD134 (antihuman Ox-40) [17], CD23 (FcεRII), and PerCP-conjugated CD8

(Becton Dickinson). Cultivated cells were washed once in RPMI with 10% AB + serum before staining. 200 µL cell suspension ( $1 \times 10^6$  cells/mL) was treated with 5 µL conjugated antibody for 30 min on ice and subsequently washed once in CellWASH (Becton Dickinson) before being read on a FACSCalibur instrument (Becton Dickinson).

50 000 events per sample were collected in an FSC/SSC plot set on lymphocytes. The TCR-Vβ families were gated among the CD4+ cells in the lymphoblast region. The acquired data were analysed using the Cellquest (Becton Dickinson) software program.

#### Cytokine production

IFNγ, IL-4 and IL-5 titres were measured in supernatants from separate untreated and bacampicillin-treated (200 µg/mL) 2 mL cultures,  $2 \times 10^6$  cells/mL. Cultures stimulated with PPD or PHA (phytohemagglutinin) served as positive controls. After 24, 48, 72, 96 and 120 h, 1 mL of the supernatant was sampled and stored at –70 °C. After sampling, the cell cultures were always restored with 1 mL of complete RPMI 1640 without antigen.

#### ELISA

Cytokine titres were measured by the BIOTRAK cellular communication assay ELISA system (Amersham, UK) according to the description of the manufacturer. The optical density was read on an Anthos htII automated microplate reader (Anthos labtec instruments, Salzburg, Austria). The mean concentration of each sample duplicate was calculated from the standard curve using the Anthos htII software.

#### Statistical methods

The sensitivity and specificity of LTT and skin tests were calculated as described earlier [18].

## Results

#### Skin tests (patch and prick test)

From the 39 patients tested, a total of 33 showed a positive reaction in at least one of the skin tests Table 2. Thus, the sensitivity of skin test was 85% when clinical history was used as a reference.

#### Type I reactions

Among the 16 patients with pure type I reactions, 11 (69%) had a positive patch test, eight of them were not prick tested. Three patients with a positive patch test were also prick

**Table 2.** Results from LTT and skin tests

| Allergic reaction<br>Subject | LTT*         |                 |                           |                 | Skin tests |            |
|------------------------------|--------------|-----------------|---------------------------|-----------------|------------|------------|
|                              | No Ag<br>cpm | PPD<br>cpm (SI) | Bacampicillin<br>cpm (SI) | Conc.†<br>µg/mL | Patch test | Prick test |
| Type I                       |              |                 |                           |                 |            |            |
| 293                          | 645          | 360299 (559)    | 57405 (89)                | 50              | +          | ND         |
| 315                          | 7259         | 12616 (2)       | 39199 (5)                 | 200             | –          | +          |
| 320                          | 1114         | 93230 (84)      | 28964 (26)                | 200             | +          | –          |
| 323                          | 1621         | 49617 (31)      | 39877 (25)                | 50              | +          | ND         |
| 324                          | 1508         | 417115 (277)    | 23072 (15)                | 50              | +          | +          |
| 331                          | 2024         | 26769 (13)      | 22264 (11)                | 200             | +          | ND         |
| 391                          | 4212         | 505676 (120)    | 10951 (3)                 | 100             | –          | +          |
| 397                          | 6346         | 156342 (25)     | 204976 (32)               | 200             | +          | ND         |
| 398                          | 6945         | 252803 (36)     | 129872 (19)               | 200             | +          | +          |
| 405                          | 4071         | 141527 (35)     | 89969 (22)                | 200             | +          | ND         |
| 409                          | 6729         | 446817 (66)     | 89496 (13)                | 200             | –          | +          |
| 418                          | 27040        | 259830 (10)     | 37856 (1.4)               | 100             | +          | ND         |
| 456                          | 2679         | 303056 (113)    | 47686 (18)                | 200             | +          | ND         |
| 484                          | 2961         | 125042 (42)     | 19839 (7)                 | 200             | +          | ND         |
| 291                          | 733          | 331292 (452)    | 1466 (2)                  | 100             | –          | –          |
| 378                          | 4509         | 234967 (52)     | 35170 (8)                 | 100             | –          | –          |
| Type IV                      |              |                 |                           |                 |            |            |
| 263                          | 12617        | 331504 (26)     | 214489 (17)               | 100             | +          | ND         |
| 264                          | 1521         | 55188 (36)      | 5628 (4)                  | 50              | +          | ND         |
| 267                          | 343          | 18480 (54)      | 7203 (21)                 | 12.5            | +          | ND         |
| 281                          | 1924         | 516803 (269)    | 59644 (31)                | 50              | +          | ND         |
| 304                          | 3226         | 30634 (10)      | 41938 (13)                | 200             | +          | ND         |
| 313                          | 7578         | 324814 (43)     | 221278 (29)               | 200             | +          | ND         |
| 325                          | 2560         | 345092 (135)    | 66816 (26)                | 100             | +          | ND         |
| 359                          | 3010         | 183226 (61)     | 8428 (3)                  | 200             | +          | ND         |
| 400                          | 2593         | 252680 (97)     | 44081 (17)                | 200             | +          | ND         |
| 401                          | 4891         | 259582 (53)     | 140861 (29)               | 200             | +          | ND         |
| 421                          | 1150         | 455327 (396)    | 300035 (261)              | 100             | +          | ND         |
| 423                          | 4740         | 1340650 (283)   | 23700 (5)                 | 200             | +          | ND         |
| 439                          | 44410        | 289087 (6)      | 164317 (4)                | 200             | +          | ND         |
| 445                          | 726          | 139514 (192)    | 1960 (2.7)                | 100             | +          | ND         |
| 453                          | 1815         | 280849 (155)    | 8712 (5)                  | 100             | +          | ND         |
| 454                          | 1126         | 241867 (215)    | 17003 (15)                | 100             | +          | ND         |
| 316                          | 1248         | 446772 (358)    | 2621 (2.1)                | 6.25            | –          | –          |
| 438                          | 17668        | 273659 (16)     | 52943 (3)                 | 200             | –          | ND         |
| 452                          | 1105         | 591978 (536)    | 2431 (2.2)                | 100             | –          | ND         |
| Type I and type IV           |              |                 |                           |                 |            |            |
| 271                          | 3236         | 303005 (93)     | 216812 (67)               | 200             | +          | +          |
| 306                          | 3779         | 67166 (18)      | 90696 (24)                | 50              | +          | ND         |
| 379                          | 1684         | 285131 (169)    | 7746 (5)                  | 50              | –          | –          |
| 433                          | 1350         | 585315 (434)    | 5265 (4)                  | 25              | +          | ND         |
| Healthy controls             |              |                 |                           |                 |            |            |
| TL92                         | 10176        | 246124 (24)     | 15785 (1.6)               | 100             | ND         | ND         |
| TL134                        | 39214        | 248101 (6)      | 44312 (1.1)               | 200             | ND         | ND         |
| TL141                        | 6245         | 381586 (61)     | 27478 (4.4)               | 200             | ND         | ND         |
| TL63                         | 4031         | 302134 (75)     | 4474 (1.1)                | 200             | ND         | ND         |
| TL53                         | 3079         | 327179 (106)    | 3849 (1.2)                | 200             | ND         | ND         |
| TL136                        | 16373        | 349352 (21)     | 29799 (1.8)               | 50              | ND         | ND         |

Table 2. continued

|                  | LTT*         |                       |                           |                 | Skin tests |            |
|------------------|--------------|-----------------------|---------------------------|-----------------|------------|------------|
|                  | No Ag<br>cpm | PPD<br>cpm (SI)       | Bacampicillin<br>cpm (SI) | Conc.†<br>µg/mL | Patch test | Prick test |
| Healthy controls |              |                       |                           |                 |            |            |
| JP               | 376          | 13134 ( <b>35</b> )   | 282 (0.8)                 | 200             | ND         | ND         |
| MMS              | 2365         | 415556 ( <b>176</b> ) | 1253 (0.5)                | 200             | ND         | ND         |
| MH               | 4656         | 184079 ( <b>40</b> )  | 1862 (0.4)                | 200             | ND         | ND         |
| TL79             | 3617         | ND                    | 9766 (2.7)                | 200             | ND         | ND         |

c.p.m. = counts per minute, SI = Stimulation index, + = positive, - = negative, ND = not done. \*Positive SI values ( $\geq 3$ ) are given in bold. †Test concentration inducing maximal proliferation.

tested, two of them had a positive and one had a negative result. Three patients were patch test negative and prick test positive. Two patients showed no reaction in either skin test.

#### Type IV reactions

Among the 19 patients with pure type IV reactions, 16 had a positive patch test. The remaining three patients were patch test-negative.

#### Type I and type IV reactions

Three of the four patients with a clinical history of both type I and type IV allergy showed a positive patch test. One patient was negative in both patch and prick test.

#### Lymphocyte transformation test (LTT)

A total of 34 of the 39 patients tested were LTT-positive to bacampicillin (Table 2). Thus, the sensitivity of LTT was 87% when clinical history was used as a reference. Fourteen

of the 16 patients with a pure type I allergy and 16 of the 19 patients with pure type IV allergy had a positive LTT result. All four subjects with combined type I and type IV allergy were LTT-positive.

One of the 10 healthy subjects without any previous exposure to bacampicillin was LTT-positive (SI = 4.4) resulting in a specificity of 90%. Thirty-four of the 39 patients (87%) were positive in both LTT and skin test. Six patients with a clinical history of allergy to bacampicillin had a negative skin test. Three of them were LTT-positive. On the other hand, five patients were LTT-negative and two of them were skin test positive. Thus, the combined sensitivity of LTT and skin tests was 92%, using clinical history as a reference. All patients tested, except no. 315, were LTT-positive to PPD, Table 2.

The LTT results of the six patients retested for cytokine profiles and flow cytometry analysis are shown in Table 3. As shown by microscopy, the number of lymphoblasts increased by a factor of at least 22 (from 0.4 to 9%) in cultures treated with bacampicillin compared to untreated cultures.

Table 3. Results of the second LTT in six patients tested for cytokine profile, and immunophenotype of lymphoblasts

| Type of allergy<br>(Patient no.) | No Ag<br>c.p.m. | % blasts* | SEB<br>c.p.m. (SI) | % blasts | Bacampicillin<br>c.p.m. (SI) | % blasts |
|----------------------------------|-----------------|-----------|--------------------|----------|------------------------------|----------|
| I (397)                          | 1873            | 3.7       | 56346 (30)         | 61       | 19685 (10)                   | 18.7     |
| I (405)                          | 1083            | <0.3      | 56289 (52)         | 25       | 14926 (14)                   | 8.4      |
| I (456)                          | 802             | 1.7       | 99847 (124)        | 58       | 18458 (23)                   | 22.4     |
| I (484)                          | 682             | <0.3      | 65395 (96)         | 45.4     | 10106 (15)                   | 8        |
| IV (263)                         | 917             | 0.4       | 126750 (138)       | 51       | 27940 (30)                   | 9        |
| IV (421)                         | 308             | <0.3      | 77789 (252)        | 44.7     | 30644 (99)                   | 13       |

\*300 lymphocytes were counted on each cell preparation.

**Table 4a.** IFN $\gamma$ 

| Time, h | Subject |      |      |      |      |      |
|---------|---------|------|------|------|------|------|
|         | 397*    | 405* | 456* | 484* | 263† | 421† |
| 24      | 0       | 0    | 0    | 0    | 0    | 0    |
| 48      | 4       | 0    | 17   | 0    | 0    | 4    |
| 72      | 6       | 0    | 9    | 0    | 13   | 9    |
| 96      | 1       | 0    | 8    | 0    | 44   | 24   |
| 120     | 1       | 0    | 3    | 0    | 41   | 32   |

**Table 4b.** IL-4

| Time, h | Subject |        |       |       |       |       |
|---------|---------|--------|-------|-------|-------|-------|
|         | 397*    | 405*   | 456*  | 484*  | 263†  | 421†  |
| 24      | 0 (0)   | 0 (0)  | 0 (0) | 0 (0) | 0 (0) | 0 (0) |
| 48      | 0 (0)   | 7 (4)  | 0 (0) | 0 (0) | 0 (0) | 0 (4) |
| 72      | 0 (0)   | 13 (2) | 0 (0) | 0 (0) | 0 (0) | 0 (0) |
| 96      | 0 (0)   | 19 (4) | 0 (0) | 0 (0) | 0 (0) | 0 (0) |
| 120     | 0 (0)   | 7 (4)  | 0 (0) | 5 (1) | 0 (0) | 0 (0) |

**Table 4c.** IL-5

| Time, h | Subject |        |        |       |           |         |       |        |       |
|---------|---------|--------|--------|-------|-----------|---------|-------|--------|-------|
|         | 397*    | 405*   | 456*   | 484*  | 263†      | 421†    | TL56‡ | JP‡    | MH‡   |
| 24      | 0 (0)   | 0 (0)  | 0 (0)  | 0 (0) | 0 (0)     | 0 (0)   | 1 (1) | 0 (0)  | 0 (0) |
| 48      | 0 (0)   | 0 (5)  | 0 (0)  | 0 (0) | 11 (0)    | 0 (0)   | 2 (2) | 0 (0)  | 0 (0) |
| 72      | 56 (0)  | 0 (0)  | 16 (0) | 5 (0) | 87 (0)    | 10 (0)  | 1 (2) | 0 (0)  | 0 (0) |
| 96      | 78 (0)  | 37 (0) | 20 (0) | 4 (0) | > 245 (0) | 68 (0)  | 2 (2) | 0 (0)  | 0 (0) |
| 120     | 32 (0)  | 65 (3) | 7 (0)  | 3 (0) | > 245 (0) | 120 (0) | 1 (2) | 2 (19) | 0 (0) |

\*Rhinitis, †ACD, ‡healthy control. ' > ' = maximal measurable cytokine concentration with the assay. *In vitro* cytokine responses to bacampicillin (200  $\mu$ g/mL) expressed in pg/mL. The cell cultures were exposed to bacampicillin for 24 h after which the first supernatant sample (1 mL) was removed. 1 mL of cultivation medium, without antigen, was then added to the cell pellet and the following supernatant was collected after another 24 h. The procedure was repeated at 48 h, 76 h, 92 h and 120 h. IL-4 and IL-5 in untreated cultures are shown in brackets, IFN- $\gamma$  was not detected in untreated cultures.

### Cytokine profiles

Bacampicillin-treated lymphocyte cultures from the two retested patients with ACD showed an IFN $\gamma$  production that peaked at 96 h or later. The four retested patients with rhinitis showed no or low IFN $\gamma$  production which peaked at 48 h or 72 h, Table 4a. Two of them responded with IL-4 production. No IL-4 was detected in cell cultures from the patients with ACD, Table 4b. IL-5 could be measured in lymphocyte cultures from all patients, irrespective of clinical response and the responses peaked at 72 h or later.

Three of the healthy control subjects were also tested for IL-5 but only one of them showed a marginal IL-5 production without any distinct peak, Table 4c. All patients showed IFN $\gamma$ , IL-5 and IL-5 production in response to the positive control substances PPD and PHA, data not shown.

### Immunophenotyping of lymphoblasts and determination of activation markers

The bacampicillin-induced blasts were predominantly CD4+ comprising 25–50% of the total blast population

**Table 5.** Immunophenotyping of SEB- and bacampicillin-induced lymphoblasts, and resting lymphocytes

| Subject | Resting (unstimulated) |            |                 |            | SEB (10 ng/mL) |            |                 |            | Bacampicillin (200 µg/mL) |            |                 |            |
|---------|------------------------|------------|-----------------|------------|----------------|------------|-----------------|------------|---------------------------|------------|-----------------|------------|
|         | CD4 +<br>%             | CD8 +<br>% | CD19+/20 +<br>% | Panγδ<br>% | CD4 +<br>%     | CD8 +<br>% | CD19+/20 +<br>% | Panγδ<br>% | CD4 +<br>%                | CD8 +<br>% | CD19+/20 +<br>% | Panγδ<br>% |
| 397*    | 60.1                   | 12.9       | 3.0             | 3.2        | 71.5           | 15.2       | 4.1             | 1.6        | 38.9                      | 5.2        | 8.8             | 2.3        |
| 405*    | 51.7                   | 23.1       | 2.6             | 3.2        | 46.2           | 30.6       | 5.8             | 3.2        | 32.8                      | 14.9       | 14.8            | 3.8        |
| 456*    | 45.7                   | 25.4       | 4.4             | 3.0        | 55.1           | 31.5       | 2.4             | 7.6        | 49.5                      | 7.4        | 7.7             | 4.8        |
| 484*    | 45.6                   | 17.4       | 3.8             | 2.0        | 51.9           | 21.7       | 5.8             | 2.4        | 33.5                      | 9.7        | 12.9            | 3.1        |
| 263†    | 63.1                   | 10.2       | 7.3             | 4.1        | 67.1           | 13.2       | 5.5             | 11.0       | 44.0                      | 7.8        | 12.5            | 9.9        |
| 421†    | 38.4                   | 17.6       | 5.0             | 8.4        | 47.9           | 27.1       | 4.5             | 1.7        | 24.9                      | 14.2       | 9.6             | 3.2        |

\*Rhinitis, †ACD

as compared to 5–15% CD8 +, 8–15% CD19+/20 + and 2–10% γδ + blasts. As expected, the majority of the SEB-induced blasts (46–72%) were CD4 + and the relative number of CD8 + blasts was increased as compared to resting untreated cells. However, there was no difference in the relative amount of CD19+/CD20 + cells or γδ + cells between ACD and rhinitis patients, Table 5. An activation of CD4 + lymphocytes was also verified by an increased expression of the early T-cell activation marker CD134 in bacampicillin treated cultures from four of five tested patients, Fig. 1(a). An increase in CD23 on B-cells was observed in bacampicillin treated cell cultures from both ACD and rhinitis patients, Fig. 1(b).

#### TCR-Vβ expression on bacampicillin-induced lymphoblasts

The most frequent TCR-Vβ family in untreated cultures was Vβ2 and constituted approximately 8% of the CD4 + lymphocyte population in all six patients investigated Fig. 2(a). After stimulation with bacampicillin, the following TCR-Vβ families constituted more than 8% of the CD4 + lymphoblasts induced by bacampicillin: Vβ2, Vβ3, Vβ5.1 and Vβ14, Fig. 2(b). This observed increase in certain Vβ families was individual, Table 6. Vβ11 was excluded from the figures since less than 0.93% of the cells belonged to this family. SEB induced predominantly Vβ3 + (10–34%), Vβ12 + (9–14%), Vβ14 + (7–13%) and Vβ17 + (23–34%) blasts in all patients, as expected with this superantigen [19].

#### Discussion

The sensitivity of LTT was 87% when a clinical history of allergy to bacampicillin was used as a reference. Corresponding sensitivity of skin tests was 85%. It should be noted that 69% of the patients with type I reactions had positive patch tests and that only 19% were patch test-negative/prick

test-positive. Patch testing, the conventional skin test for identification of type IV allergy, has also earlier proven useful in patients with type I allergies [20,22]. In a recent paper on allergy to beta-lactams, skin tests were suggested to be false negative to a greater extent than LTT. In that study the sensitivity of LTT was 74% as compared to 64% for skin test when analysed against clinical history [7]. Contrasting, in our study LTT and skin tests were equally sensitive and the

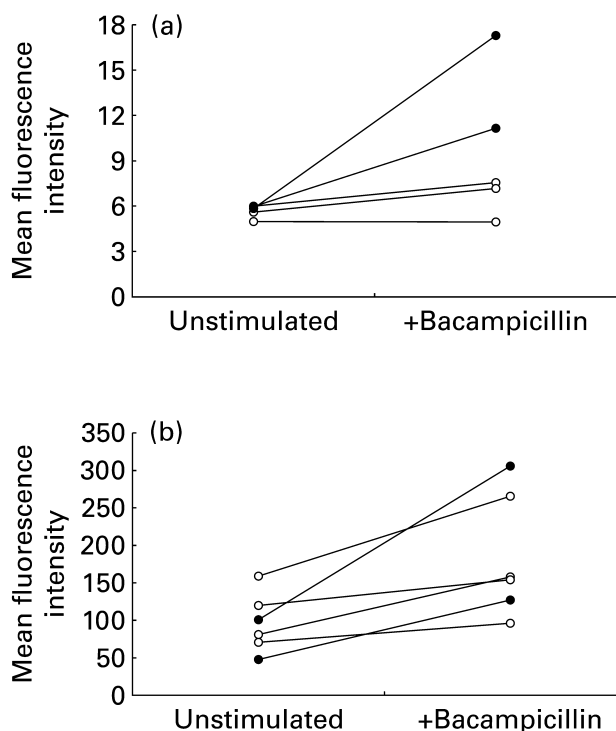


**Fig. 1.** Expression of activation markers in 5 day cultures without antigen or with bacampicillin, 200 µg/mL. ○ = patients with type I allergy, ● = patients with type IV allergy. (a) CD134 expression on CD4 + lymphocytes. One patient with rhinitis (456) was not tested. (b) CD23 expression on CD19+/CD20 + lymphocytes.

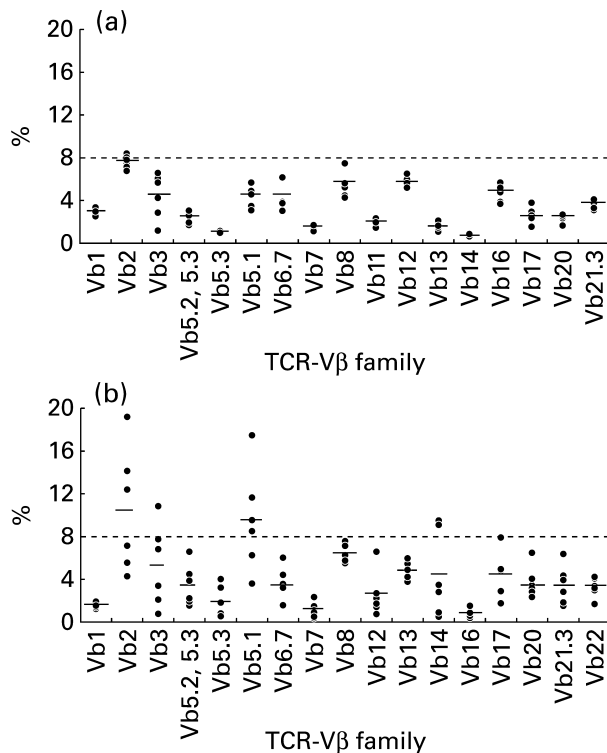


**Fig. 2.** TCR-V $\beta$  distribution of CD4 $^{+}$  lymphocytes from all patients ( $n = 6$ ). Mean values of each TCR-V $\beta$  family are indicated by bars. (a) % of indicated TCR-V $\beta$  family expressed by CD4 $^{+}$  resting lymphocytes in 5 day culture without antigen exposure. (b) % of indicated TCR-V $\beta$  family expressed by lymphoblasts in 5 day culture treated with bacampicillin, 200  $\mu$ g/mL.

test results correlated in 87% of cases. The obtained specificity of LTT with bacampicillin, 90%, is in agreement with a recent study on phenoxymethylpenicillin where the specificity of LTT was 96% [4]. In the present study, LTT and skin tests reached a sensitivity of 92% when used in combination. Accordingly, LTT and skin tests are valuable as complementary tests to identify bacampicillin-allergic subjects.

**Table 6.** TCR-V $\beta$  families constituting >8% of the CD4 $^{+}$  bacampicillin-induced lymphoblasts (in bold)

| Subject | V $\beta$ 2<br>% | V $\beta$ 3<br>% | V $\beta$ 5.1<br>% | V $\beta$ 14<br>% |
|---------|------------------|------------------|--------------------|-------------------|
| 397*    | <b>12.4</b>      | 7.7              | <b>9.6</b>         | 3.5               |
| 405*    | <b>14.1</b>      | 0.8              | <b>17.5</b>        | <b>9.5</b>        |
| 456*    | 7.1              | 3.4              | 6.2                | 2.8               |
| 484*    | 4.3              | 2.1              | <b>8.5</b>         | <b>9.1</b>        |
| 263†    | 5.6              | <b>10.8</b>      | <b>11.7</b>        | 0.5               |
| 421†    | <b>19.2</b>      | 6.6              | 3.6                | 0.9               |

\*Rhinitis, †ACD.

The dominant phenotype of bacampicillin-induced lymphoblasts was CD4 $^{+}$  in all patients tested. Blasts with a phenotype of CD8 $^{+}$ , CD19/20 $^{+}$  or  $\gamma\delta^{+}$  were also present, however, without difference between rhinitis- and ACD-patients. Otherwise, CD8 has been suggested as being the predominant phenotype of penicillin-specific T cells in peripheral blood [23]. In that particular study, T-cell clones isolated by limiting dilution were investigated. However, it is unknown if the results from such a random selection of cells could be extrapolated to the *in vivo* situation since penicillin specific CD4 $^{+}$  T-cell clones have also been identified [9–24]. An increase in  $\gamma\delta$  cells has earlier been observed in human skin lesions from patients with ACD to DNCB [25]. Further, lidocaine-specific  $\gamma\delta^{+}$  T-cell clones have been prepared from the peripheral blood of patients with positive patch tests to this drug [26] but penicillin-specific  $\gamma\delta$ -cells have not earlier been identified. Our findings suggest the possible presence of penicillin-specific lymphocytes also among  $\gamma\delta^{+}$  cells and B-lymphocytes.

This is the first time that drug-induced lymphoblasts from primary cultures have been TCR-V $\beta$  characterized. Earlier studies have used either the total lymphocyte population [27] or cell lines or clones [9,10,24,26,28]. We found that bacampicillin-induced CD4 $^{+}$  lymphoblasts had a heterogeneous TCR-V $\beta$  repertoire dominated by V $\beta$ 2, V $\beta$ 3, V $\beta$ 5.1, or V $\beta$ 14. The occurrence of V $\beta$ 5.2 + T cell lines [10], and V $\beta$ 2 + and V $\beta$  5.1 + T-cell clones [9] has earlier been reported for benzylpenicillin-specific CD4 $^{+}$  lymphocytes. The two latter families were also identified in the present study suggesting that flow cytometric screening of primary induced lymphoblasts could be useful as a tool to identify V $\beta$  families that harbor antigen-specific cells.

Th1 cells (in type IV allergy) secrete mainly IL-2 and IFN- $\gamma$ , Th2 cells (in type I allergy) produce IL-4, IL-5, IL-6 and IL-10 while Th0 cells secrete a variety of these cytokines. However, these cytokine patterns are reversible [29]. When T-cell clones are used for subsequent analysis of critical cell functions, such as cytokine production, the clonal expansion procedure may have affected their behaviour. That is, by using polyclonal activators such as PMA, by adding IL-2 and by repeating the stimulation cycles, the original cytokine pattern produced by the antigen-specific cells may have changed. Penicillin G-specific T-cell clones have earlier been found to secrete a highly heterogeneous cytokine pattern [9], a typical property of Th0 cells. In that study, IFN- $\gamma$  was secreted from T-cell clones generated from patients with type I allergy even though a Th2 response would have been expected. In our study, IFN- $\gamma$  production was associated with type IV but not with type I allergy. However, IL-5 was found in bacampicillin-treated cell cultures from both ACD and rhinitis patients. Similar

amounts of IL-5, approximately 200 pg/mL, have earlier been detected in primary cultures stimulated with benzylpenicillin [30]. In our study, the IL-5 release detected in cell cultures from the patients was not an unspecific effect of the drug *per se*. Neither IL-5 nor IFN $\gamma$  or IL-4 (data not shown) could be found in cultures from healthy subjects. Thus, IL-5 could possibly be used as a general marker for bacampicillin induced allergy. This Th2 related *in vitro* response, detected in both ACD and rhinitis patients, was further confirmed by an up regulation of CD23 on B-cells and CD134 on CD4+ cells. Furthermore, the two patients who exhibited the highest IL-5 titres also showed the strongest expression of CD134, an activation marker known to promote B-cell help [31].

Hitherto, studies dealing with drug-specific cytokine release in primary cultures have used cumulative values [24,26,27,30]. The cytokine responses in our study were induced after an optimal *in vitro* exposure to bacampicillin on day 1. On days 2–5 a part of the supernatant was subsequently removed and replaced by culture medium without antigen. It is interesting to conclude that 24 h with an optimal antigen concentration is enough to stimulate cytokine release that still can be measured after 120 h. Thus, we have shown that a continuous antigen concentration is not necessary to induce cytokine production *in vitro*.

In conclusion, *in vitro* responses from primary PBMC cultures could be used to distinguish type IV from type I allergy by measuring cytokine profiles, and seem useful to screen for TCR-V $\beta$  families that may harbour antigen-specific cells. This cell culture system could possibly be used as an alternative to T-cell lines or clones since it may better reflect the *in vivo* situation. Further, LTT and skin tests are valuable as complementary tests in the diagnosis of allergy to bacampicillin since the combined use increases the sensitivity.

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