Original Article

Interleukin-4 and interferon-γ are possible allergic markers in pediatric patients with β-lactam hypersensitivity

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Abstract

Background: β-lactam agents are known to elicit T-cell-mediated immune responses that play a central role in the onset of allergic reactions, but the involvement of specific type of cytokines in drug allergy remains largely unexplored in humans. **Objectives:** This study was undertaken to investigate the role of cytokines involvement in pediatric patients with β-lactam hypersensitivity and to determine whether involvement of cytokines in drug-mediated reactions are important for the perspective of allergic patient's management. **Methods:** β-lactam-induced hypersensitivity reactions in eighty pediatric patients were determined by clinical manifestations and skin prick or intradermal testing. Production of T-helper (Th) type-1 cytokine interferon (INF)-γ, Th-2 cytokine interleukin (IL)-4, regulatory T-cell cytokine IL-10, and other cytokines IL-6 and IL-12 were determined by sandwich ELISAs. **Results:** Diagnosis of β-lactam allergy was confirmed in 53 pediatric patients. IL-4 secretion in patients' sera was significantly higher as compared with healthy controls ($P < 0.05$). However, INF-γ level in patients' sera was significantly lower as compared with controls ($P < 0.05$). No significant alterations were found in the protein secretion of IL-10, IL-12, and IL-6 in allergic patients as compared with controls ($P > 0.05$). **Conclusion:** We conclude that IL-4 is specific marker for the diagnosis of β-lactam-induced hypersensitivity. Moreover, IL-4 in combination with INF-γ is more sensitive for the diagnosis of these reactions. This study also concludes that both IL-4 and INF-γ may play an active role in the onset of allergic reactions against β-lactam antibiotics.

Key words: Hypersensitivity, interferon-γ, interleukin-4, pediatric patients, β-lactam

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Introduction

Allergic reactions to β-lactams are the most common cause of drug reactions mediated by specific immunological mechanisms, where Immunoglobulin E (IgE) and T-cells play a role in the onset of allergic reactions.[1,2] Although the production process of β-lactams has improved over the years, the number of allergic reactions associated with them has not been decreased, probably because the number of subjects exposed to β-lactams antibiotics has risen.[1] The great diversity of chemical structures of β-lactams antibiotics

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available has resulted in the generation of a larger number of hapten-carrier conjugates, which can be recognized by immune system.[1,3] Modifications in the use and choice of β-lactams antibiotics have caused epidemiological alterations in the population of patients allergic to such antibiotics and justify a revision of part diagnostic guidelines.[2,3] Depending on the time interval between administration of drugs and the occurrence of the allergic reactions, it has now been well classified as drug-mediated allergic reactions as immediate or delayed. Immediate reactions to β-lactams antibiotics may be clinically classified as urticaria or anaphylaxis, occurring within 1 h of antibiotic administration.[1,4] A detailed, clinical history of the patient's reaction is required, including the symptoms the time elapsed between administration of the β-lactams antibiotics and the appearance of symptoms as well as the time elapsed between the clinical reaction and the allergic evaluation and also previous reactions to these antibiotics.[1,5]

Recent cellular and molecular features of presentation of β-lactams drugs to specific T-cells and T-cell responses to haptenic drugs have been the subject of growing interest. The clinical picture of T-cell-mediated drug hypersensitivity reactions is very heterogeneous, and different T-cell subsets are supposed to be involved.[2,6] The diagnosis of T-cell-mediated drug hypersensitivity reactions is a major challenge in daily clinical practice as patch, prick, and intracutaneous tests often do not yield positive results even in patients with a clear history of drug hypersensitivity.[6-9] The main challenge for diagnosis of drug hypersensitivity reaction is to confirm that the symptoms were caused by drug hypersensitivity and to identify the culprit drug. In the recent years, it has been shown that *in vitro* T-cell proliferation and activation tests can be applied to the diagnosis of drug hypersensitivities,[2] but the role of T-cells still not fully discovered. In allergic disorders such as atopic dermatitis and hyper-IgE syndrome, inappropriate expression of T-cells-mediated cytokine production was well reported.[7] Published literature on atopic subjects reveals conflicting conclusions as to the nature of the specific defect in cytokine production.[7,10] In many studies, interferon (IFN)-γ production was reported to be essentially normal in atopic subjects,[11] whereas in others, it was markedly reduced[12] or elevated.[13] Interleukin (IL)-4 production was usually[14] but not always[15] reported as substantially higher. In some studies, neither IL-4 nor IFN-γ production was different,[7,16] whereas in others, synthesis of both cytokines was affected, with IFN-γ inhibited and IL-4 elevated[7,17] relative to the responses elicited in normal controls. Some of these discrepancies may reflect different etiologies for related but distinct hypersensitivity states, i.e., atopic dermatitis versus allergic rhinitis.[7,10-17] However, the variation frequently observed by different groups studying the same disease may also stem from the fact that many studies have been carried out with very small numbers of subjects.[7] Despite the availability of modern approaches, these discrepancies in cytokines productions under allergic disorders have not been fully clarified. Therefore, it is very important to determine a systematic comparison of different cytokines produced after drug administration in patients showing drug hypersensitivity.

Here, we examined the role of cytokines in allergic patients that showed β-lactam hypersensitivity. β-lactam-induced hypersensitivity was confirmed by clinical manifestations diagnosis and skin prick test (SPT) or intradermal test (IDT). Our novel results show that IL-4 was significantly higher and interferon (INF)-γ was significantly lower in patients' sera as compared with their respective healthy controls' sera. However, no significant change was found in the production of IL-6, IL-12, and IL-10 in allergic patients' sera and healthy controls' sera. Our data conclude that INF-γ and IL-4 may pay a role in the onset of hypersensitive reactions after β-lactam drugs administration. Moreover, data also conclude that analysis of these cytokines in patients' sera is useful for the diagnosis of drug hypersensitivity.

Methods

The study was performed in the College of Medicine, Qassim University, KSA, between January 2013 and March 2015. The study group included 80 Saudi children, 36 females and 44 males. Out of them, 53 children showed β-lactam positive hypersensitivity and were considered as patients. However, other sixty children showed β-lactam negative hypersensitivity and were considered as controls, and they had no history of any type of hypersensitivity. All participants were randomly selected from Qassim University affiliated hospitals. Informed consent was obtained from the parents of all participants. The racial or ethnic compositions of the patients were comparable with those of the controls. The complete demographical and general laboratorial characterizations of enrolled subjects are summarized in Table 1. The study was carried out in accordance with the code of ethics of the World of Medical Association (Declaration of Helsinki) for humans and was approved by the ethical review board committee, College of Medicine, Qassim University.

SPT and IDT were performed with the soluble forms of the suspected β-lactams. SPTs were carried out first and if negative, IDTs were also performed. In all children, testing was carried out using the major and minor determinants of penicillin, penicillin G (PG), amoxicillin, ampicillin, benzathine penicillin, benzylpenicillin, ceftriaxone, cefaclor, and cefotaxime and other β-lactams. SPT and IDT were performed with major determinants undiluted (penicilloyl poly-L-lysine [PPL]) (Diater, Madrid, Spain), minor determinants

undiluted (MDM) (Diater), PG 10,000 U/mL (Sandoz GmbH, Kundl, Austria), ampicillin (25 mg/mL) (ampicillin sodium equivalent to 500 mg ampicillin activity; Bristol-Myers Squibb Co., New York, NY, USA), amoxicillin (25 mg/mL) (Hymox Forte in powder form; Biocheme Spimaco, Kingdom of Saudi Arabia), and for cephalosporins 1–2 mg/mL. Histamine phosphate (10 mg/mL) and saline solution were used as positive and negative controls, respectively. IDTs were performed using 1/100, 1/10, and 1/1 concentrations. Both SPT and IDT were assessed 20 min after application and again after 24–48 h for any delayed reaction to occur. The results were considered positive if a wheal and flare reaction larger than the negative control was present during SPT or 3 mm higher than the injected papule in IDT.

Venous blood samples from the control subjects and patients were collected and sera were separated and stored in small aliquots at −70°C until analyzed further. Samples with gross hemolysis and lipemia were excluded. Serum levels of IL-4, IL-6, IL-10, and IL-12 were measured by specific Avi-Bion human IL sandwich ELISA kits according to the manufacturers' instructions (Ani Biotech Oy, Orgenium Laboratories, Vantaa, Finland). The minimal detection level of IL-4, IL-6, IL-10, and IL-12 by this method was 15.6, 7.8, 3.9, and 3.6 pg/mL, respectively. However, INF-γ level in the serum samples was measured by human INF-γ sandwich ELISA kit as per instructions described (GenWay Biotech, CA, USA). The minimal detection level of INF-γ using this method was 46 pg/mL. Total IgE levels were measured in the serum samples by human IgE-specific sandwich ELISA according to the manufacturers' instructions (BioCheck, Inc., Foster City, CA, USA) with a sensitivity of 1–500 U/mL.

Table 1: Demographical and general laboratorial characterization of enrolled subjects

Variable	Patients (positive β-lactam)	Controls (negative β-lactam)	*P*
Number of subjects (male/female)	80 (44/36)	60 (33/27)	-
Age (year)	11.3±2.0	11.3±1.7	1.000
Height (cm)	138.8±9.9	138.8±7.9	1.000
Weight (kg)	33.6±7.3	32.9±7.3	0.780
Head circumference (cm)	52.9±1.1	52.4±1.3	0.147
BMI	17.3±2.1	17.02±1.3	0.737
Hemoglobin (g/dl)	10.3±0.8	11.7250±1.2	0.000*
WBCs (×10³/mm³)	7.2±1.93	7.8850±2.4	0.336
Platelets (×10³/mm³)	242.8±47.1	232.5000±52.4	0.519
Segmented (%)	56.6±9.9	57.2±10.04	0.850
Lymphocytes (%)	34.7±6.5	33.2±9.5	0.576
Monocytes (%)	3.4±1.95	2.8±1.9	0.370
Eosinophil (%)	3.7±2.25	2.65±2.3	0.157
RBCs (×10⁶/mm³)	4.08±0.49	4.3150±0.5	0.170

*Statistically significant *P* value. Results are expressed as mean±SD. BMI: Body mass index; WBCs: White blood cells; IgE: Immunoglobulin E; RBCs: Red blood cells; SD: Standard deviation

Statistical analysis was performed using the SPSS Program version 16.0 (SPSS Inc., Chicago, IL, USA). $P < 0.05$ was considered statistically significant. Results are expressed as mean ± standard deviation (SD).

Results

A total of eighty pediatric patients underwent a drug allergy evaluation for β-lactam agent's hypersensitivity. Diagnosis of β-lactam-induced drug allergy was confirmed in 53 patients (66.2%). Demographic and all common laboratory details are collected and summarized in Table 1. Diagnosis of allergy caused by β-lactam agents was confirmed and the data are shown in Figure 1a. The leading culprit drug was ceftriaxone in 30% patients, followed by cefotaxime in 15% patients, ampicillin in 13.5%, cefaclor in 12.5%, amoxicillin + clavulanic acid in 12.5%, ampicillin in 8.75% patients, benzathine penicillin in 5%, and benzylpenicillin in 2.5% patients. Clinical manifestations of β-lactam antibiotic-induced allergic reactions are shown in Figure 1b. Maculopapular rashes were found in 40% patients, followed by maculopapular rashes with angioedema in 26% patients. However, urticaria was found in 17% patients; erythema and anaphylactic shock were also found in 10% and 7% of patients, respectively. Among the 53 confirmed positive patients using skin testing, we had 21 patients positive by SPT and 32 positive by IDT. Among the different reagents tested, we had 26 patients positive to PPL, 8 patients to MDM, 15 patients to PG, 18 patients to ampicillin, 28 patients to amoxicillin, and 39 patients to cephalosporin [Table 2].

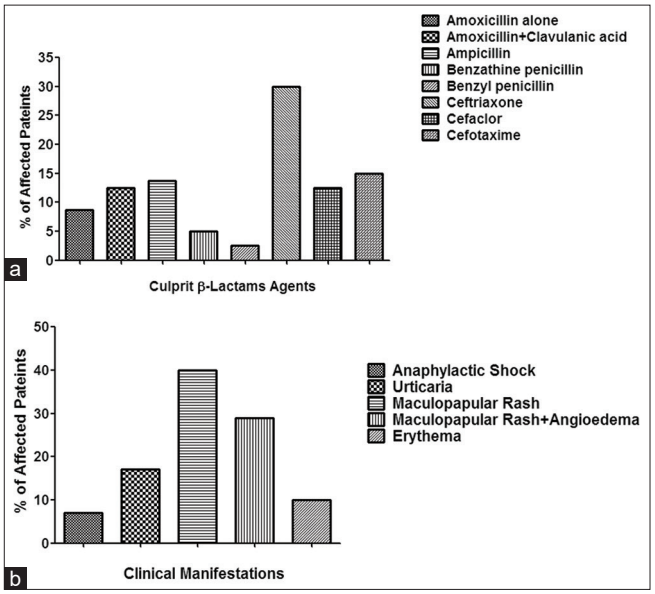

Figure 1: (a) Culprit β-lactam agents of the original reaction in affected pediatric patients. Each histogram represents percentage of the total. (b) Clinical presentation of the drug reactions in affected patients. Each histogram represents percentage of the total

Table 2: Skin testing data on studied subjects reacting to more than one agents

	PPL		MDM		PG		Ampicillin		Amoxycillin		Cephalosporin	
	SPT	IDT	SPT	IDT	SPT	IDT	SPT	IDT	SPT	IDT	SPT	IDT
Positive subjects (%)	9 (11)	17 (21.3)	5 (6)	3 (3.8)	4 (5)	11 (13.8)	6 (7.5)	13 (16.3)	12 (15)	16 (20)	17 (21)	22 (27.5)
Negative subjects (%)	71 (89)	63 (78.7)	75 (94)	77 (96.2)	76 (95)	69 (86.2)	74 (92.5)	67 (83.7)	68 (85)	64 (80)	63 (79)	58 (72.5)

Values are expressed as number of subjects (%). PPL: Penicilloyl poly-L-lysine; MDM: Minor determinant mixture; PG: Penicillin G; SPT: Skin prick test; IDT: Intradermal testing

In an attempt to find out the role of cytokines in pathogenesis of allergic patients which are showing hypersensitivity against β-lactam antibiotics, this report determines the serum levels of INF-γ, IL-4, IL-6, IL-12, and IL-10 in allergic patients (n = 53) and their results were compared with healthy controls (n = 60). The levels of IL-4 were determined and were found to be significantly higher in patients as compared with healthy controls (P < 0.0001) [Figure 2a]. The average (±SD) of IL-4 level in patients' sera and control human sera was 178.0 ± 110.2 and 48.1 ± 35.1 pg/mL, respectively. In contrast, the serum levels of INF-γ were significantly lower in patients as compared with healthy controls (P < 0.05). The average (± SD) of INF-γ levels in patients' sera and control human sera was 161.9 ± 20.9 and 210.1 ± 45.5 pg/mL, respectively [Figure 2b]. Whereas the levels of IL-6, IL-12, and IL-10 in the serum samples of allergic patients were found to be statistically similar as compared with their respective healthy controls (P > 0.05). The average (±SD) of IL-6 in patients' sera and control human sera was 221.3 ± 135.9 and 263.05 ± 140.50 pg/mL, respectively [Figure 3a], whereas the average (± SD) of IL-12 in patients' sera and controls' sera was 113.6 ± 91.8 and 106.0 ± 37.6 pg/mL, respectively [Figure 3b]. Moreover, the average (± SD) of IL-10 in patients and controls sera was 98.17 ± 62.8 and 66.3 ± 36.6 pg/mL, respectively [Figure 3c]. To further validate our results under allergic conditions, we also determined the levels of IgE under the same experimental conditions. Interestingly, the serum levels of IgE were found to be significantly higher in patients as comparison with healthy controls (P < 0.001). The average (± SD) of IgE levels in 53 patients' sera and 60 control sera was 166.0 ± 114.8 and 56.79 ± 101.8 IU/mL, respectively [Figure 4].

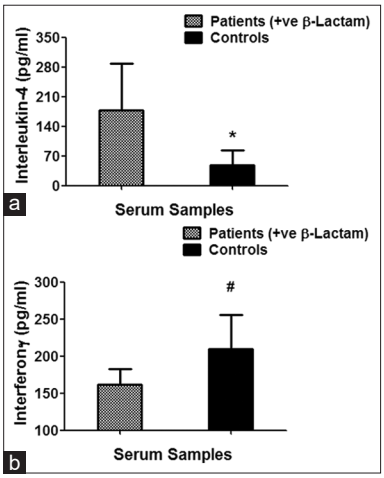

Figure 2: (a) Interleukin-4 levels in serum samples of pediatric patients with β-lactam hypersensitivity. *P < 0.0001 versus controls. (b) Interferon-γ levels in serum samples of pediatric patients with β-lactam hypersensitivity. #P < 0.05 versus controls. Histograms show the mean ± standard deviation

Discussion

Skin drug hypersensitivity reactions are one of the most common types of adverse reactions to drug therapy, with an overall incidence rate of 2–3% in hospitalized patients.[18] Almost any medicine can induce cutaneous reactions, and drug-induced hypersensitivity rate is continuously increasing in all over the world. Cutaneous drug reactions in the pediatric patients have a positive impact on patients' current and future care options.[19] The quick detection and treatment of cutaneous drug reactions, plus identification of the causative agent, are important for preventing the progression of the drug reaction, preventing additional exposures, and ensuring the appropriate use of medications for both the current condition and others as the patient ages.[19] Although most drug-related cutaneous hypersensitivity reactions are not serious, some are severe and potentially life-threatening. Serious reactions include angioedema, erythroderma, Stevens–Johnson syndrome, and toxic epidermal necrolysis.[20] Drug hypersensitivity can also occur as part of a spectrum of multiorgan involvement, for example, in drug-induced systemic lupus erythematosus.[21] As with other types of drug reactions, the pathogenesis of hypersensitivity reactions may be either immunological or nonimmunological.[22] The pathophysiology and the underlying mechanisms of many drug-induced hypersensitivity reactions remain poorly understood and poorly correlated with clinical symptoms.[18-23] Therefore, carefully evaluations of all drug-associated hypersensitivity reactions are required. In this study, we have tested eighty pediatric patients for β-lactam agent's hypersensitivity. Out of them, 66.2% patients showed drug allergy. The leading culprit drug was found to be ceftriaxone, followed by cefotaxime, ampicillin, cefaclor, combination of amoxicillin and clavulanic, amoxicillin alone, benzathine penicillin, and benzylpenicillin. Despite this positivity's of β-lactam antibiotics toward the tested children, the most reliable approach for evaluating allergic reactions to β-lactam antibiotics was found to be a detailed description of the symptoms which can be obtained from the patients' parents or quite often from witnesses. Another source of information was the clinical manifestations.

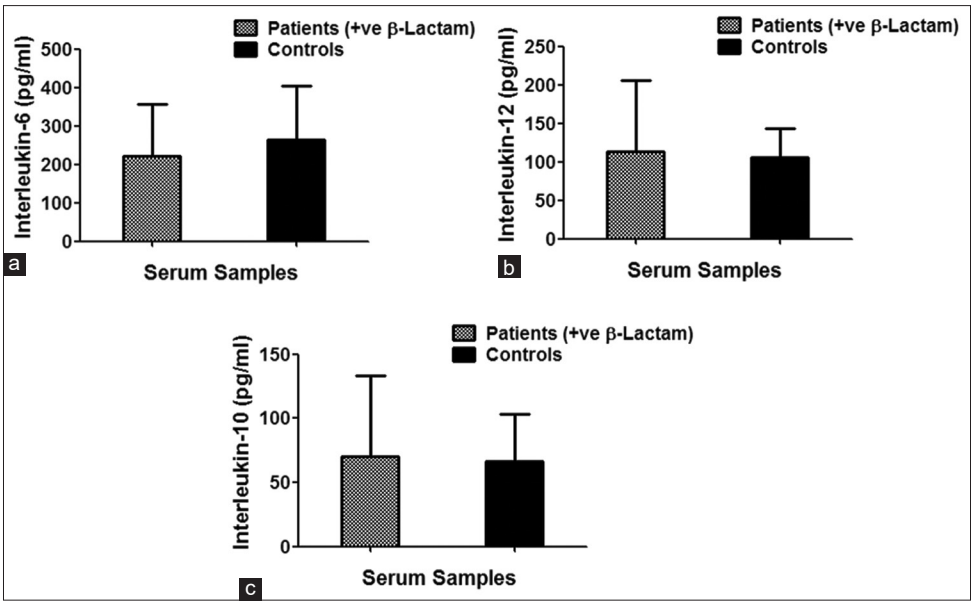

Figure 3: (a) Interleukin-6 levels in serum samples of pediatric patients with β-lactam hypersensitivity. Patients versus controls, $P > 0.05$. (b) Interleukin-12 levels in serum samples of pediatric patients with β-lactam hypersensitivity. Patients versus controls, $P > 0.05$. (c) Interleukin-10 levels in serum samples of pediatric patients with β-lactam hypersensitivity. Patients versus controls, $P > 0.05$. Histograms show the mean ± standard deviation

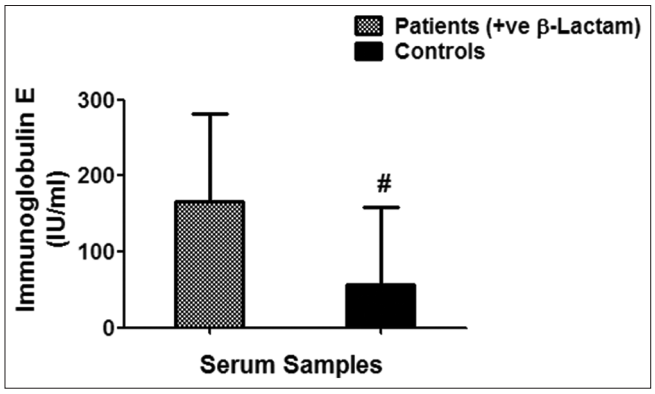

Figure 4: Immunoglobulin E levels in serum samples of pediatric patients with β-lactam hypersensitivity. #$P < 0.001$ versus controls. Histograms show the mean ± standard deviation

In this study, maculopapular rashes were found in 40% patients, followed by maculopapular rashes in combination with angioedema in 26% patients. Whereas, urticaria was found in 17% patients; erythema and anaphylactic shock were in 10% and 7% of patients, respectively. Among the 53 confirmed positive patients using skin testing, we had 21 patients positive by SPT and 32 positive by IDT. Among the different reagents tested, we had 26 patients positive to PPL, 8 patients to MDM, 15 patients to PG, 18 patients to ampicillin, 28 patients to amoxicillin, and 39 patients cephalosporin. This high positivity percentage of β-lactam antibiotics to the patients warned the health professionals for the danger of drug allergy in the treatment of patients.

One of the most potentially useful tools in drug-induced toxicology is the assessment of cytokines, the molecules responsible for regulating a variety of processes including immunity and inflammation.[7,24] The particular profile of cytokine production may provide important information regarding the nature of hypersensitivity. Due to their critical role in all phases of the immune response, cytokines are important experimental parameters in investigating the induction and elicitation phases of various hypersensitivity responses, including allergic inflammation.[24] At present, it is well established that cytokines play an important role in many drug-mediated hypersensitivity reactions, ranging from mild rashes to life-threatening reactions.[7-15,24] However, the potential for specific cytokine (s) to elicit hypersensitivity responses or to contribute to allergic pathogenesis remains largely unexplored in human. Therefore, in this study, we have made an attempt to investigate the role of cytokines in the pathogenesis of pediatric patients which showed hypersensitivity against β-lactam agents. Our data show the serum levels of INF-γ were significantly lower in patients as compared with healthy controls ($P < 0.05$), whereas the levels of IL-4 were found to be significantly higher in patients as compared with healthy controls ($P < 0.0001$). Moreover, the levels of IL-6, IL-12, and IL-10 in the serum samples of allergic children were found to be almost similar as were in the healthy controls ($P > 0.05$). To further validate our results under allergic conditions, we also determined the levels of IgE under the same experimental conditions. Interestingly, the serum levels of IgE were found to be significantly higher in patients as comparison with healthy controls ($P < 0.001$). These data are in full agreement with the view that T-helper type 2 (Th2) cytokines are thought to be involved in cutaneous reactions due to their ability to modulate antibody responses.[9] It includes IL-4 which promotes B-cell

proliferation, differentiation, and isotype class-switching to IgE antibodies.[25] In contrast, IFN-γ, a Th1 cytokine, negatively regulates antibody-mediated reactions.[26] Not only are these, our results further supported by many studies performed in different allergic patients.[7,27] As for example, in allergic rhinitis, patients markedly increased IL-4 and weaker IFN-γ production were reported as compared with nonatopic subjects.[7] Moreover, dysfunctionality at mRNA level of IL-4 and INF-γ was also reported in peripheral lymphocytes, derived from patients of β-lactam hypersensitivity.[8] Collectively, the data argue from this study and studies discussed above, clearly indicating that IL-4 and INF-γ play a role in the onset of hypersensitivity reactions against β-lactam agents.

To conclude, the measurement of IL-4 is a very specific marker for the diagnosis of β-lactam-induced hypersensitivity. Moreover, determination of IL-4 in combination with INF-γ may be more sensitive tool for the determination of these drug-induced reactions.

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Conflicts of interest

There are no conflicts of interest.

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