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A PRELIMINARY STUDY OF IL-5, IL-6 AND IFN-GAMMA SERUM LEVELS IN PATIENTS WITH GENERALIZED DRUG ERUPTIONS

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ABSTRACT

PURPOSE. The aim of the present study was to investigate the serum levels of IL-5, IL-6 and IFN-gamma in patients with generalized drug eruptions of various clinical patterns.

PATIENTS AND METHODS. Six patients with generalized drug eruptions and fifty one healthy subjects serving as controls were included. The causal relationship between drugs and adverse reactions was evaluated with the Naranjo algorithm. Blood samples for cytokine assay were collected at the time of full blown rash. Commersial solid-phase sandwich enzyme immunoassay for human, IL-5, IL-6 and IFN-gamma (Quantikine® from R&D Systems, Inc) was used.

RESULTS. Serum IL-5 was elevated only in one patient with DRESS syndrome. Serum IL-6 was insignificantly elevated in patients with drug eruptions compared to controls. There were no significant differencies in serum IFN-gamma levels between patients and controls.

DISCUSSION. The combined investigation of cytokines in drug eruptions may contribute to a better understanding of their pathogenesis, and consequently to exact diagnosis and treatment. For definite conclusions more patient cases and repeated blood sampling are required.

Key words: drug eruption, DRESS syndrome, interleukins, IL-5, IL-6, IFN-gamma.

INTRODUCTION

Drug eruptions (DEs) are the most frequent type of adverse drug reactions and the majority of these reactions are supposed to be allergic in nature. DEs can mimic a wide range of dermatoses. Diagnosing DEs may be difficult when a clear causal relationship to drug intake is absent. Recently determination of certain serum cytokine levels in drug and viral exanthema has been proposed to differentiate between these morphologically similar skin eruptions (1, 2). Various cytokine patterns have been identified in morphologically different DEs and this reflects the differences in their pathogenesis.

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Cytokines are low molecular weight, soluble proteins produced by immunocompetent cells that communicate with other cells to regulate immune function. Human interleukin-5 (IL-5) is produced mainly by Th_2 lymphocytes and is considered to be a key player in the coordination and orchestration of eosinophil-based inflammatory processes (3). Increased plasma IL-5 levels have been reported in some patients with hypersensitivity syndrome (4) and skin allergy exanthema (2). IL-6 is a major cytokine produced by human macrophages and is thought to play a central role in allergic inflammation (5). Elevations of IL-6 have been described in serum of patients with toxic epidermal necrolysis (6), drug-induced maculopapular eruption (7) and drug-induced hypersensitivity syndrome (8). IFN-gamma is produced by lymphocytes activated by specific antigens. In addition to having antiviral activity, it has important immunoregulatory functions. High IFN-gamma production has

been found in type IVa delayed type hypersensitivity reactions (9).

Maculopapular exanthema and urticaria are common clinical presentation of DEs. DRESS syndrome (drug reaction with eosinophilia and systemic symptoms) or "hypersensitivity syndrome" is a severe, acute drug reaction defined by the presence of fever, skin eruption and systemic findings - enlarged lymph nodes, abnormal liver function, renal impairment, pulmonary or cardiac infiltrates, haematological abnormalities, mainly hypereosinophilia and lymphocytosis (10). Its exact incidence is not known due to variability in presentation (11) and lack of a specific and sensitive diagnostic test (12).

The aim of the present study was to investigate the serum levels of IL-5, IL-6 and IFN-gamma in patients with generalized DEs of various clinical patterns.

PATIENTS AND METHODS

Patients

Six patients (2 males and 4 females) with an age range from 27 to 67 years with generalized DEs were included. The clinical presentation was DRESS syndrome (3 cases), maculopapular exanthema (2 cases), acute urticaria (1 case). DRESS syndrome was diagnosed in 3 female patients with clinical features appearing 3 to 4 weeks after administration of carbamazepne. In all patients the clinical manifestation was similar: a maculopapular rash progressing to exfoliative erythroderma, fever, and lymphadenopathy. Leukocytosis, atypical lymphocytes and liver dysfunction were also observed. Diagnosis was based on the criteria of Kennebeck (13) and the exclusion of infections, neoplastic and collagen vascular diseases with microbiological, serological, immunological and instrumental investigations. Systemic symptoms accompanying the DE were detected in all patients with DRESS syndrome (fever and lymph node enlargement in all cases; liver injury in 2 cases). One of the patients with maculopapular exanthema was found to have fever and malaise in the course of the eruption.

The causal relationship between drugs and adverse reactions was evaluated with the Naranjo algorithm (14).
Serving as controls were 51 healthy subjects (43 females and 8 males) with age ranging from 19 to 71 years.

Blood sampling

Blood samples for cytokine assay were collected as part of a routine laboratory check-up of patients with a generalized DEs at the time of full blown rash. Blood was collected and allowed to clot at room temperature for 30 minutes before spinning. Serum was aliquoted and stored at -20^{0}C prior to assessment.

Cytokine Assay

Commersial solid-phase sandwich enzyme immunoassay for human, IL-5, IL-6 and IFN-gamma (Quantikine® from R&D Systems, Inc) was used. The sensitivity of the kits was above 3 pg/ml for IL-5, above 0,7 pg/ml for IL-6 and above 8 pg/ml for IFN-gamma. ELISA tests were calibrated using recombinant human cytokine standards. Samples were assayed in duplicate.

Statistical analysis

Variables were tested for normality using Shapiro–Wilk test. Since plasma cytokone concentrations were not distributed in a Gaussian manner the Mann-Whitney test was used to test for differences between patients and controls. A value of $p<0.05$ was considered statistically significant. Analyses were performed using SPSS for Windows version 9.0.

RESULTS

Patients with generalized DEs included 6 cases of various clinical presentation **(table 1)**. The causality of the adverse reactions were rated as "probable" in cases 2, 3 and 6 and as "possible" in the rest of the cases.

The serum concentration of IL-5 was elevated only in one patient with DRESS syndrome **(table 2).** IL-5 was not detected in controls.

IL-6 was insignificantly elevated **(table 3)** in patients with DEs (median 1.8 pg/ml; interquartile range 0.3-6.02 pg/ml) compared to controls (median 1.2 pg/ml; interquartile range 0.4-2.4 pg/ml).

There were no significant differencies (p=0,17) in serum IFN-gamma levels (table 3) in patients with generalized DEs (median 1.25 pg/ml; interquartile range 1.07-1.85 pg/ml) compared to controls (median 1.65 pg/ml; interquartile range 1.3-2.15 pg/ml).

Table 1. *Patients with generalized drug eruptions – demographics, clinical diagnosis, causality*

Case N	Sex	Age	Diagnosis	Systemic findings	Eliciting drug	Naranjo score
1	F	28	DRESS s-me	Fever, lymph node enlargement, liver injury	CBZ	4
2	F	50	DRESS s-me	Fever, lymph node enlargement, liver injury	CBZ	5
3	F	34	DRESS s-me	Fever, lymph node enlargement	CBZ	5
4	F	27	MPE	Fever, malaise	Ketoprofen	3
5	M	67	MPE	-	Metamizole	4
6	M	33	Urticaria	-	Metamizole	5

F – female; M – male; MPE – maculopapular exanthema; CBZ - carbamazepine

Table 2. *Patients with generalized drug eruptions – main laboratory findings*

Case N	Sex	Age	Diagnosis	Peak eosinophilia	IL-5 (pg/ml)	IL-6 (pg/ml)	IFN-γ (pg/ml)
1	F	28	DRESS s-me	5.0 x 10^9/L	0	0.3	1.1
2	F	50	DRESS s-me	5.4 x 10^9/L	32.1	16.6	2.6
3	F	34	DRESS s-me	1.5 x 10^9/L	0	0.3	1.2
4	F	27	MPE	-	0	1.3	1.0
5	M	67	MPE	-	0	2.5	1.6
6	M	33	Urticaria	-	0	2.3	1.3

F – female; M – male; MPE – maculopapular exanthema; CBZ - carbamazepine
*Blood sample for cytokine determination was taken 4-7 days after peak eosinophilia in cases 1 and 3, and 3 days before peak eosinophilia in case 2.

Table 3. *Serum IL-6 and IFN-gamma in patients and controls*

	IL-6 (pg/ml)	IFN-γ (pg/ml)	Significance
Patients with DEs	median 1.8; IQR 0.3 - 6.02	median 1.25; IQR 1.07 - 1.85	p=0.59
Controls	median 1.2; IQR 0.4 - 2.4	median 1.65; IQR 1.3 - 2.15	p=0,17

IQR - interquartile range

DISCUSSION

The present study found 1 out of 3 patients with DRESS syndrome to have elevated serum IL-5. DRESS syndrome is a rare, acute drug reaction, with variable clinical presentation, lack of strict diagnostic critera and underreporting of cases. The blood sample of the patient with high IL-5 was taken before the peak of eosinophilia and this confirms the results of Choquet-Kastylevsky (4). It seems that IL-5 is detectable only early in the course of the disease and decreases rapidly. In patients with exanthematous and urticarial eruptions serum IL-5 was not detectable. This is in concordance with the results of others investigating IL-5 in patients with DEs without eosinophilia (4). The determination of serum IL-5 in the absence of eosinophilia may be helpful in providing evidence for a drug-unduced allergic reaction (1). Because high serum levels of IL-5 have been detected in some studies in drug-induced, as well as in viral exanthema (2) only the combined determination of IL-5 and IFN-gamma (usually elevated in viral infections) may be relevant in differentiating allergic from viral etiology.

Confirming data from literature (1, 2) serum IFN-gamma was not elevated in the study patients. It is notable that IFN-gamma remains undetectable even when a DE like DRESS syndrome has been linked to reactivation of a viral infection like HHV-6 infection (8). The serum level of IFN-gamma seems to be

informative in cases when the drug-induced allergic reaction may be accompanied by viral reactivation.

The elevation of serum IL-6 in this series did not reach statistical significance. IL-6 has been found in high levels in patients with severe drug reactions. A series of patients with maculopapular eruptions also showed elevation of serum IL-6 (7). The different sensitivity of the assays and the relatively small number of patients in this study may explain the difference in these results. It is also possible that being produced by macrophages as well as by T lymphocytes, the detectable level of IL-6 might have been reached earlier in the course of the allergic reaction and has decreased at the time blood was collected.

The diverse clinical presentation of delayed hypersensitivity reactions depends on the T cells and cytokine pathways involved (9). The combined investigation of cytokines in DEs may contribute to a better understanding of their pathogenesis, and consequently to exact diagnosis and treatment. Determination of IL-5 before the peak of eosinophilia could be a useful laboratory tool in the diagnostic armamentarium of DRESS syndrome - a severe DE for which strict diagnostic criteria are still lacking. For definite conclusions more patient cases and repeated blood sampling are required.

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