

***In Vitro* Release of Interferon-gamma and Macrophage Migration Inhibition Factor in Drug-induced Urticaria and Angioedema**

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T-cells are involved in the pathogenesis of cutaneous drug reactions. T-cell phenotype and cytokine release pattern *in vivo* and *in vitro* might correlate with the type of immune response involved in cutaneous drug reactions. *In vitro* release of interferon- γ and macrophage migration inhibition factor (MIF) from peripheral blood lymphocytes, following *in vitro* challenge with the suspected unmodified drugs, was studied in 12 patients with drug-induced urticaria and/or angioedema and in two group-matched controls. The occurrence of positive interferon- γ and MIF responses was significantly higher in patients with drug-induced urticaria and/or angioedema than in controls. The sensitivity and specificity of the interferon- γ test (50% and 92%, respectively) were similar to that of the MIF test (58% and 96%, respectively). Percentage agreement between both tests was 80.9 (kappa = 0.76). *In vitro* release of interferon- γ and MIF in drug-induced urticaria and/or angioedema suggests a drug-specific immune response, and may implicate the drug as a possible inducer of the reaction. Key words: adverse drug reactions; cutaneous drug reactions; cytokine; *in vitro* test.

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^aT-lymphocytes are involved in the pathogenesis of cutaneous drug reactions (CDRs). The phenotype of the responding T-cells, and their cytokine pattern, *in vivo* and *in vitro*, might correlate with the type of immune response which evolves after antigen contact (1). Metabolic predisposition to drug hypersensitivity reactions (2) and a possible role of cytochrome P450-dependent enzymes in the formation of the nominal antigen, which is recognized by antigen-specific T-cells, have been reported (3). *In vivo* and *in vitro* studies have implied that drug-specific CD8+ T-cells producing a Th1-type cytokine pattern are preferentially activated in delayed-type hypersensitivity (DTH) reactions, such as morbilliform and bullous exanthems, whereas drug-specific CD4+ Th2-type cells are preferentially activated in immediate-type hypersensitivity reactions, such as urticarial exanthems (1, 4, 5).

In vitro tests based on drug-related T-cell activity, such as macrophage migration inhibition factor (MIF) release (6–12), a correlate of DTH and cell-mediated immunity (CMI) (13, 14), have been used by us as a diagnostic tool in various types of adverse drug reactions. Interferon-gamma (IFN- γ), is one of the important mediators of DTH responses, mainly

acting to activate macrophages (15). Furthermore, rIFN- γ has been shown to have MIF-like activity (16).

The aim of the present study is to evaluate *in vitro* drug-induced IFN- γ release as compared to MIF release in patients with the clinical diagnosis of drug-induced urticaria and/or angioedema.

MATERIAL AND METHODS

Patients

Twelve patients (4 males, 8 females; age range 3–78 years; mean age 41 years) with the clinical diagnosis of acute drug-induced urticaria and/or angioedema were referred to our laboratory for *in vitro* identification of the offending drug(s). The duration of the reactions ranged from several days (in most of the patients) to 3 weeks. None of the patients suffered from physical urticaria. Prior to the development of that adverse drug reaction the patients were treated with 15 different drugs (1–4 drugs per patient), for variable periods of time, which were equally suspected as being the inducers of the adverse reactions.

Each patient was subjected to two *in vitro* tests, measuring the release of IFN- γ and MIF from peripheral blood lymphocytes, following *in vitro* challenge with the suspected drugs. A total of 21 drug tests were performed. None of the patients was being treated with systemic corticosteroids at the time of the *in vitro* tests, which were performed after the acute phase of the reaction. Clinical data with respect to follow-up, including drug withdrawal, treatment and remission of the eruption, are presented in Table I. Challenge tests were not performed due to medicolegal considerations. Nor were skin tests performed.

Controls

Forty patients (14 males, 26 females; age range 30–85 years; mean age 52 years) treated with 12 drugs without known adverse reactions, served as a group-matched control for the IFN- γ test. A total of 64 drug tests were performed in this group of controls. These included tests for acetylsalicylic acid (9 tests), diclofenac sod. (9 tests), captopril (7 tests), enalapril maleate (6 tests), nifedipine (7 tests), atenolol (5 tests), thyroxine sod. (5 tests), glibenclamide (5 tests), quinidine bisulfate (3 tests), ranitidine (5 tests), paracetamol (2 tests) and allopurinol (1 test).

Ninety-five patients (43 males, 52 females; age range 30–88 years; mean age 67 years), treated with 11 drugs without known adverse reactions, served as a second group-matched control for the MIF test. A total of 95 drug tests were performed in this group of controls. These included tests for diclofenac sod. (10 tests), nifedipine (6 tests), propranolol (17 tests), atenolol (2 tests), dipyrone (10 tests), paracetamol (7 tests), hydrochlorothiazide (15 tests), furosemide (7 tests), isosorbide (8 tests), quinidine sulphate (10 tests) and dipyridamole (3 tests).

MIF test and IFN- γ test

Lymphocytes from heparinized venous blood were separated by Ficoll Hypaque gradient centrifugation. The MIF test was performed according to the technique described previously by Livni et al. (6). It involved incubation of the patients' peripheral blood lymphocytes and peritoneal guinea-pig macrophages, packed into plain capillary tubes, in the presence of the respective unmodified drug for 24 h. The tested drug was dissolved in the appropriate solvent, and finally diluted in a med-

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Table I. Clinical data and distribution of IFN- γ and MIF responses for suspected drugs in 12 patients with drug-induced urticaria and angioedema

Pat	Age/ sex	Reaction	Suspected drug	IFN- γ response (% IFN- γ increase)	MIF response (migration index)	Drug withdrawal	Treatment	Remission
1	40/F	Urticaria/angioedema (acute)	Dipyrone	negative (0)	negative (1.0)	+	A, AH, CS	+
			Paracetamol	negative (0)	negative (0.91)	+		
2	30/F	Urticaria (acute)	Oestradiol and oestriol	positive (1054)	positive (0.60)	+	AH, CS	+
3	30/M	Urticaria (acute)	Indomethacin	negative (0)	negative (1.0)	–	AH, CS	+
4	2.5/M	Urticaria/angioedema (acute)	Paracetamol	negative (0)	negative (1.0)	+	AH, CS	+
5	63/F	Urticaria (acute)	Bezafibrate	negative (0)	negative (0.92)	–	CS	+
			Enalapril maleate	negative (0)	positive (0.71)	–		
			Oestriol	negative (0)	negative (0.96)	–		
6	48/F	Urticaria (acute)	Captopril	positive (34)	positive (0.63)	U	AH	U
7	36/F	Urticaria (acute)	Progesterone	negative (0)	positive (0.69)	+	AH, CS	+
8	52/M	Urticaria (acute)	Acetylsalicylic acid	positive (180)	negative (1.08)	U	AH	U
9	59/F	Urticaria/angioedema (acute)	Acetylsalicylic acid	positive (150)	positive (0.77)	+	CS	±
			Paracetamol	negative (0)	negative (0.96)	–		
10	15/M	Urticaria/angioedema (acute)	Paracetamol	positive (52)	positive (0.73)	+	AH, CS	+
			Acetylsalicylic acid	positive (108)	positive (0.77)	+		
			Captopril	negative (0)	negative (1.15)	+		
			Thyroxine sod.	negative (0)	negative (1.05)	+		
11	43/F	Angioedema (acute, recurrent)	Alprazolam	negative (0)	negative (0.92)	–	AH, CS	+
12	78/F	Angioedema (acute)	Doxepin	negative (15)	positive (0.74)	+	U	U
			Clobazam	negative (0)	negative (1.0)	+		
			Selegiline HCl	positive (51)	positive (0.70)	+		
Total pos.				6/12 patients 7/21 tests	7/12 patients 9/21 tests			

A = adrenalin; AH = oral anti-histamine preparations; CS = systemic corticosteroids; U = unknown.

*Self-administration of acetylsalicylic acid resulted in reappearance of the adverse reaction.

[percentage agreement in the results of IFN- γ and MIF responses: 80.9%; kappa = 0.76].

ium containing fetal calf serum (FCS). Drugs were used as antigens in three concentrations that did not inhibit free macrophage migration. Macrophage migration in the presence of the drug was compared with that in the absence of the drug, and the ratio was expressed as a migration index. A migration index of 0.80 or less at one of the drug concentrations was considered to be a positive MIF response.

The lymphocytes for the IFN- γ test were twice washed with M-199 Hank's medium, containing antibiotics (penicillin – 100 u/ml, streptomycin – 100 μ g/ml), and suspended in the same medium containing 15% FCS. The lymphocytes were cultured in test tubes, in concentrations of 2×10^6 cells/ml of M-199 Hank's medium (plus 15% FCS), containing Phytohemagglutinin P (purified – Difco Laboratories, Detroit, MI, USA) at the concentration of 40 μ g/ml and each drug, or with medium alone. Incubation was done for 24 h in 37°C and 5% CO₂. Unmodified drugs dissolved in the appropriate solvents were used. The drug concentrations used were extrapolated from the known therapeutic doses. The viability of the lymphocytes incubated with these doses was tested with trypan blue. The two maximal drug concentrations, which were found to be non-toxic to the lymphocytes, were used. After incubation, the test tubes were centrifuged in 2500 rpm for 25 min at 5°C. The supernatants were collected and stored in –70°C until used. The ELISA for human IFN- γ in the supernatants was performed with the Quantikine kit (R&D Systems, USA) and according to the manufacturer's instructions. The standards were diluted in the same medium of the samples. All standards and samples were performed in duplicate. IFN- γ release in the supernatants of peripheral blood lymphocytes was reflected by the percentage of IFN- γ increase calculated according to the following formula: % IFN- γ increase = $100 \times (\text{IFN-}\gamma \text{ (pg/ml) with the drug} - \text{IFN-}\gamma \text{ (pg/ml) with medium alone}) / \text{IFN-}\gamma \text{ (pg/ml) with medium alone}$.

The results were based on the maximal IFN- γ release in one of the drug concentrations used. A positive IFN- γ test response to drugs was determined as the mean percentage increase of IFN- γ measured in the supernatants of controls + 2 SD. A positive IFN- γ test response for 11 drugs, consisting of diclofenac sod., captopril, enalapril maleate, nifedipine, atenolol, thyroxine sod., glibenclamide, quinidine bisulfate, ranitidine, paracetamol and allopurinol was determined as 27% IFN- γ increase. A positive IFN- γ test response to acetylsalicylic acid was determined as 88% IFN- γ increase.

Statistical analysis

The results of the IFN- γ and MIF tests obtained in patients and controls were compared and analysed using the chi-square test for the differences in proportions between the study groups. The kappa test was used on the percentage agreement of reaction between the two tests compared.

RESULTS

The occurrence of positive IFN- γ recorded in 6 of the 12 patients with drug-induced urticaria and/or angioedema was significantly higher than that recorded in 3 of 40 controls ($p < 0.005$). Similarly, the occurrence of positive MIF responses recorded in 7 of the patients was significantly higher than that recorded in 4 of 95 controls ($p < 0.005$). Considering the adverse drug reaction as the "gold standard", the sensitivity and specificity of the IFN- γ test (50% and 92%, respectively) were similar to those of the MIF test (58% and 96%, respectively).

The distribution of IFN- γ and MIF responses for suspected drugs used by the 12 patients with drug-induced urticaria/angioedema (21 tests) is presented in Table I. Positive IFN- γ responses were recorded for 7/21 of the drug tests, whereas positive MIF responses were recorded for 9/21 of the drug tests. Coexistence of positive IFN- γ and positive MIF responses was observed in five patients (nos. 2, 6, 9, 10, 12)

for five different drugs (acetylsalicylic acid, paracetamol, captopril, selegiline HCl and a combination of oestradiol and oestriol). Coexistence of negative IFN- γ and negative MIF responses was observed in eight patients (nos. 1, 3, 4, 5, 9, 10, 11, 12) for nine different drugs (dipyron, paracetamol, indomethacin, bezafibrate, captopril, oestriol, thyroxine sod., alprazolam and clobazam). Negative IFN- γ and MIF test results for drugs taken were observed in four patients (nos. 1, 3, 4, 11). Dissociation between IFN- γ and MIF test results was observed in four patients (nos. 5, 7, 8, 12) for 4 different drugs (enalapril maleate, progesterone, acetylsalicylic acid, doxepin). The kappa value calculated for the percent agreement in the results of IFN- γ and MIF responses was 0.76, a value which represents excellent agreement (80.9%), i.e. statistically significant. In two patients (nos. 10 and 12) positive IFN- γ and/or MIF responses were recorded for two different drugs. Possible clinical relevance, based on drug withdrawal and remission of the reaction, is presented in Table I.

DISCUSSION

The present study demonstrates *in vitro* drug-induced release of IFN- γ and MIF by peripheral blood lymphocytes, challenged *in vitro* with the suspected unmodified parent drug compounds, in patients with acute drug-induced urticaria and/or angioedema.

In vitro cytokine release may have a diagnostic role in CDRs (1). MIF release in adverse drug reactions and various types of CDRs was increased in patients compared to controls (6–12), and was increased in response to suspected drugs compared to non-suspected drugs (7, 8). Recent studies related to drug-specific T-cell clones derived from the peripheral blood mononuclear cells (PBMC) of patients with CDRs, revealed the release of Th1-type cytokines, including IFN- γ , in patients with beta-lactam-induced morbilliform exanthems (4) and in patients with acute drug allergy to carbamazepine, phenytoin, allopurinol, or paracetamol (manifested by exanthem) (17). The diagnostic role of *in vitro* IFN- γ release has recently been reported in a case of carbamazepine-induced erythroderma (18), in whom following incubation of the patient's PBMC with unmodified carbamazepine, a significant high level of IFN- γ was detected in the supernatant, as compared to controls.

Release of MIF has also been reported in drug-induced IgE-mediated hypersensitivity reactions, such as IgE-mediated allergy induced by horse serum in mice (19) and immediate-type allergy to penicillin (20). In diclofenac sodium-induced urticaria (21) the positive MIF response recorded for the offending drug was associated with a positive rat mast cell degranulation test result, suggesting the coexistence of drug-induced CMI and immediate-type hypersensitivity reaction. Similarly, release of Th1-cytokines, including IFN- γ has been reported in penicillin allergy, manifested by urticaria, angioedema and exanthem (22). T-cell clones specific for penicillin G (CD4+ or CD8+) or penicillin G coupled to human serum albumin (CD4+) generated from the PBMC of the patients, produced a heterogeneous cytokine pattern, showing high amounts of IL-2, IFN- γ , TNF- α , and rather variable levels of IL-4 and IL-5.

Most commonly used drugs are simple compounds (haptens) and therefore are poor immunogens. Various mechanisms may account for the immunogenicity of the unmodified parent drug compounds in the present study (2, 4, 23–26). The underlying

mechanism responsible for the occurrence of a Th1-type cytokine release in drug-induced urticaria and/or angioedema, that primarily activates Th2 subsets, is not clear. It is suggested that polarization of T-cell responses into distinct Th1 and Th2 differentiation may depend on the immunogenicity of the antigen, antigen dose, its route of administration and the immunization protocol (27).

Accordingly, the relatively low sensitivity of IFN- γ and MIF tests recorded by us in patients with drug-induced urticaria and/or angioedema (50% and 58%, respectively) may reflect the balance between the preferential activation of Th2-type or Th1-type cells. The possible involvement of non-immunologic mechanisms in the generation of urticaria and angioedema induced by acetylsalicylic acid (aspirin) and angiotensin-converting enzyme inhibitors, such as enalapril and captopril, cannot be overlooked (28, 29). Negative IFN- γ and MIF responses may also reflect lack of antigenic metabolites *in vitro*, or non-drug etiologies involved the induction of urticaria and/or angioedema. The occurrence of positive IFN- γ and/or MIF responses for two different drugs recorded in two of the patients is not clear. Cross-reactivity between acetylsalicylic acid and paracetamol, reported in aspirin intolerance (28), may play a role in one of the patients (no. 10).

The increased IFN- γ release observed in the controls, following *in vitro* challenge of lymphocytes with the cyclooxygenase inhibitor, acetylsalicylic acid, may be related to modulatory effects of PGE₂ on the cytokine secretion profile of T-cells (30).

In vitro drug-induced cytokine release from peripheral blood lymphocytes indicates immune sensitization to the drug and does not necessarily constitute definite proof that the patient's clinical manifestations result from an immune sensitization elicited by that drug. Clinical relevance may be attributed to *in vitro* drug-induced IFN- γ and MIF release in patients with drug-induced urticaria and angioedema, based on the excellent agreement between IFN- γ and MIF test results, follow-up evaluation and previous reports (7, 8). However, as challenge tests were not performed, the usefulness of these *in vitro* tests as diagnostic tests needs further elucidation.

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