In Vitro Interferon-Gamma Release Test in Patients with Drug-Induced Pemphigus

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Abstract

Background: Drug-specific CD8+ TH1 lymphocytes have been found in the peripheral blood and involved skin of patients with drug-induced bullous exanthems.

Objectives: To determine whether the interferon-gamma release test can identify culprit drugs in pemphigus patients.

Methods: Clinical and laboratory workup for pemphigus was performed in 14 pemphigus vulgaris patients who had been exposed to drugs, and the IFNγ release test was conducted on their lymphocytes from heparinized venous blood cultured with medium, phytohemagglutinin and one of 32 drugs, or medium and phytohemagglutinin alone.

Results: Ten of the patients and 13 of the 32 drugs exhibited a positive response to the test. Eight of the 10 patients with positive IFNγ test results had a less severe course of the disease, with fast reduction in steroid dosage.

Conclusions: The findings demonstrate both the ability of the IFNγ release test to identify drugs that can induce pemphigus, and its usefulness in the diagnostic workup of pemphigus patients.

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Pemphigus is a severe bullous autoimmune disease that depends on an interaction between genetic predisposition and exogenous factors, such as drugs, nutritional factors, and others [1]. Drugs implicated in the induction of pemphigus fall into three main groups according to their chemical structure: a) Drugs containing a sulfhydryl radical (thiol drugs or SH drugs), including penicillamine, captopril, gold sodium thiomalate, 5-thiopyridoxine, pyritinol, thiamazole, thiopronine, mercaptopropionylglycine, bucillamine, penicillin and piroxicam. b) Phenol drugs, including combined phenol and thiol drugs such as pyritinol and 5-thiopyridoxine, and phenol drugs like cefadroxil, rifampin, levodopa, aspirin, heroin and pentachlorophenol. c) Non-thiol non-phenol drugs, including calcium channel blocker, angiotensin-converting enzyme inhibitors, non-steroidal anti-inflammatory drugs, dipyrone, glibenclamide, and others [2-6].

Reports of drug-induced pemphigus have multiplied over the years since penicillamine was first implicated in the disease [2,3,7]. Establishing the relationship between a suspected drug and pemphigus is complicated by the fact that most patients take more than one drug at a time, and there is a latent period ranging from a few days to more than a year between taking the drug and the appearance of the disease. Several clinical and laboratory methods have been developed to identify the connection between a suspected drug and a skin disease. Recent studies demonstrated the diagnostic potential of a test based on release of interferon-gamma from lymphocytes after exposure to a suspected drug. In this test, lymphocytes of patients are incubated with and without the drug, the level of IFNγ collected from the supernatant is measured by enzyme-linked immunosorbent assay, and the increase in IFNγ release is calculated.

This is the preferred, most widely used *in vitro* test for the diagnosis of drug allergies, with high specificity of 92% and sensitivity of 77.8% [8-11]. Previous studies showed a 74.2% agreement (kappa = 0.434) between the results of *in vitro* IFNγ release tests and *in vivo* challenge tests, reflecting an intermediate to good degree of agreement [11]. Another widely used test is the lymphocyte transformation test [8].

Based on the presence of drug-specific CD8+ TH1 lymphocytes, which produce interleukin-2 and IFNγ in the peripheral blood and involved skin of patients with drug-induced bullous exanthems [12], we speculated that the IFNγ release test might be helpful in the search for culprit drugs in pemphigus patients. In 2000 we introduced the IFNγ gamma release test as part of the routine investigations in patients suspected of having drug reactions. We present here the results of this test in a series of patients with pemphigus who had been exposed to drugs before the onset of symptoms. We were looking for a state of immune sensitization toward the suspected drug, and the identity of the responsible drug.

Patients and Methods

The study group comprised 14 patients with pemphigus vulgaris attending the dermatology department at the Tel Aviv Sourasky Medical Center who had been exposed to drugs for 3 months prior to the onset of clinical symptoms of pemphigus. The average age of the 6 men and 8 women was 54 years (range 29–90 years). In addition to the routine clinical and laboratory workup for pemphigus, the IFNγ release test was performed.

The method has been described in detail elsewhere [13]. Briefly, patients' lymphocytes from heparinized venous blood are separated by Ficoll-hypaque gradient centrifugation, and cultured for 24 hours in test tubes containing medium, phytohemagglutinin and each drug, or medium and phytohemagglutinin without the drug. Unmodified drugs dissolved in the appropriate solvents

IFNγ = interferon-gamma

are used. Following incubation for 24 hours in 5% CO_2 at 37°C, the test tubes are centrifuged at 2500 rpm for 25 min at 5°C. The supernatants are collected for the detection of IFNγ release using the ELISAtechnique (Biosource, Enco Diagnostics, Petah Tikva, Israel). IFNγ release is reflected in the percentage of its increase calculated by the formula:

% IFNγ increase = 100x (IFNγ with the drug - IFNγ with medium alone)/IFNγ with medium alone.

A positive IFNγ test response has been determined to be 30% based on past measurements.

ELISA = enzyme-linked immunosorbent assay

This study was approved by the Tel Aviv Sourasky Medical Center Ethics Committee.

Results

The results of the IFNγ release test in the pemphigus patients are presented in Table 1. Ten of the 14 patients (71%) exhibited a positive response to the test. Thirteen of the 32 drugs (41%) elicited a positive response. The number of positive drugs for each patient is summarized in Table 2.

The study disclosed eight drugs associated with pemphigus vulgaris that were not previously connected with the disease: metformin, a homeopathic agent of unknown identity, latanoprost, engerix B, disothiazide, brotizolam, famotidine, and haloperidol.

Two of the four patients with negative IFNγ test results had more severe disease, with higher and longer steroid dosage and more relapses. Eight of the 10 patients with positive IFNγ test results had a less severe course of the disease, with fast reduction in steroid dosage.

Patient #4 improved promptly after elimination of latanoprost, but relapsed a few months later when he began using the medication again. We withdrew the drug again, which brought prompt improvement. Patients #3 and 14 experienced relapses of the disease even after the suspected drugs were eliminated.

Discussion

We have demonstrated drug-induced release of interferon-gamma by peripheral blood lymphocytes of pemphigus patients when challenged *in vitro* with the suspected drugs. While most drugs that tested positive in our study are known to induce pemphigus, some are described here for the first time. The drugs are metformin, benazepril, a homeopathic drug (whose contents are unknown,

Table 1. Results of the IFNγ release test in pemphigus patients

Patient #	Age (yrs)	Gender	Drugs	IFN release test	Severity of disease
1	45	F	Metformin (Glucophage®, Abic)	+	Mild
			Benazepril (Cibacen®, Promedico)	+	
2	59	F	Colchicine (Rafa)	–	Mild
3	35	M	Homeopathic1	+	Severe
			Homeopathic2	–	
4	90	M	Furosemid (Fusid®, Teva)	–	Mild-rapid improvement with relapse when patient used the drug again
			Aspirin (Cartia®, Novolog)	–	
			Alfuzosin (Xatral®, Mediline)	–	
			Latanoprost (Xalatan®, Promedico)	+	
5	62	F	Enalapril (Convertin®, MSD)	+	Mild-rapid improvement
			Simvastatin (Simovil®, MSD)	–	
6	54	F	Chlorpyrifos	+	Mild-rapid improvement
7	57	F	Chlorpyrifos	+	Mild-rapid improvement
8	35	F	Morphine (Teva)	–	Mild
			Conjugated estrogens (premaril, dexxon)	–	
9	57	M	Haloperidol (Haldol®, Janssen)	+	Mild-rapid improvement
10	54	M	Propoxyphene+paracetamol (Algolysin Forte®, Teva)	–	Severe
			Folic acid (Rekah)	–	
			Amiloride+hydrochlorothiazide (Kaluril®, MSD)	–	
			Vit B_1+ Vit B_6 +Vit B_{12} (Tribemin®, Sam-On)	–	
11	29	F	Dipyrone (Optalgin®, Teva)	–	Mild-rapid improvement
			Paracetamol (Acamol®, Teva)	+	
12	40	M	Engerix-B® (SmithKline Beecham Healthcare)	+	Mild-rapid improvement
13	52	M	Dipyrone (Optalgin®, Teva)	–	Severe
			Paracetamol (Acamol®, Teva)	–	
14	84	F	Captopril® (Teva)	–	Severe
			Raloxifene (Evista, Lilly)	–	
			Maprotiline (Melodil)	–	
			Atenolol (Normalol, Dexxon)	–	
			Famotidine (Teva)	+	
			Disothiazide (Dexxon)	+	
			Brotizolam (Teva)	+	

Table 2. The number of positive drugs for each patient

Patient #	Positive drugs
1	2 of 2
2	0 of 1
3	1 of 2
4	1 of 4
5	1 of 2
6	1 of 1
7	1 of 1
8	0 of 2
9	1 of 1
10	0 of 4
11	1 of 2
12	1 of 1
13	0 of 2
14	3 of 7

even to the homeopath!), latanoprost ophthalmic preparation, the pesticide chlorpyrifos, haloperidol (first described here as causing pemphigus vulgaris), hepatitis B vaccination, disothiazide, brotizoman, famotidine, and paracetamol.

Glibenclamide has been described as an inducer of pemphigus [14]; we report here for the first time that metformin can also induce the disease.

The literature contains several reports of ACE inhibitors associated with pemphigus [14,15]. The ACE inhibitor enalapril was found *in vitro* to be a powerful acantholytic agent [15]. Our study adds benazepril to the ACE inhibitors associated with pemphigus. Homeopathic treatment was implicated in childhood bullous pemphigoid [16], and found by us to trigger pemphigus vulgaris. Ophthalmic preparations were reported to induce other bullous diseases [17]. In the present study we describe the first case of latanoprost-induced pemphigus vulgaris. Two cases of pemphigus induced by the pesticide chlorpyrifos were described by us recently [18]. Haloperidol, a drug belonging to the phenol group, was described only once in the literature as inducing pemphigus foliaceous [19]. We describe the first case of pemphigus vulgaris induced by this drug.

Brenner et al. [20] reported a positive macrophage migration inhibition test to a combination drug compounded of paracetamol, caffeine, chlorpheniramine maleate and phenylephrine HCl in a woman who took the drug 2 weeks prior to the appearance of the cutaneous lesions. We describe a patient with a positive IFNγ release test to paracetamol.

Immunizations have been reported to induce pemphigus [21]. Childhood bullous pemphigoid following hepatitis B immunization was reported by Erbagci [22]. Ours is the first report of a case of pemphigus vulgaris induced by hepatitis B vaccination. Erbagci [22] suggested that the hepatitis B surface antigen may function as the trigger for pemphigus by stimulating a specific antibody production that may cross-react with pemphigus antigens.

We also describe here the induction of pemphigus by disothiazide, brotizolam and famotidine. One of the proposed mechanisms to explain drug-induced pemphigus is immunological reaction with neoantigen formation. The MIF test, based on this mechanism of cellular immunity, was previously employed to identify culprit drugs in skin diseases [20,23,24]. MIF is a lymphokine released from sensitized T lymphocytes by an appropriate antigen; in this test, macrophage migration is compared in the presence and absence of a drug and the ratio is expressed as a migration index. In view of the fact that MIF and the IFNγ release test yield highly similar results [8,13], and the greater ease of performance of the latter, the IFNγ release has come to replace MIF.

The positive responses to the IFNγ release test in our series reflect a state of immune sensitization of our patients to the suspected drug, and a possible relationship between the drug and the outbreak of the autoimmune disorder. The test also helped identify the drug that caused the disease from among several the patient was taking. The relationship between the drug and the skin disease, which was previously based only on circumstantial evidence, was strengthened by the interferon release test. The prompt improvement in the disease following elimination of the suspected drug further strengthened the test's reliability. This was most impressive in patient # 4 when a "self" challenge test confirmed latanoprost as the culprit.

In patients 1 and 14 more than one drug gave positive results. Immune sensitization to a few drugs indicates that those drugs, or a preservative common to them, may play a role in the induction or exacerbation of the disease.

Our speculation that the use of interferon might be useful in determining the involvement of a suspected drug in pemphigus is strengthened by a number of basic science studies. Rico and co-workers [25] determined cytokine and cell marker expression in perilesional skin biopsies from patients with pemphigus. Immunohistochemistry and *in situ* hybridization were used to detect T helper 1 (interleukin-2, IFNγ) and Th2 (IL-4, IL-5, IL-13) protein and mRNA. Perilesional skin biopsies from patients with pemphigus were remarkable for a mixed Th1/Th2 pattern of cytokine expression, including the presence of IL-2, IFNγ and IL-4 and the absence of IL-5 and IL-13. Hertl and Merk [14] reported that immunohistochemical analysis had identified CD8+ TH1 cells as the predominant epidermal T cell subset in drug-induced bullous eruptions. These epidermal T cell clones were cytotoxic against autologous B cells upon stimulation through the T cell receptor and against epidermal keratinocytes in cytotoxicity assays.

Our findings demonstrate the ability of the IFNγ release test to identify drugs that can induce pemphigus, as well as its usefulness in the diagnostic workup of pemphigus patients. While the specificity of the *in vitro* IFNγ release test is high (above 90%), its lower sensitivity might account for negative results. Nevertheless, correlation between the test results and the clinical picture can lead to the correct diagnosis. In cases that do not improve with elimination of a drug that tests positive, other possible triggers must be sought — other drugs or other exogenous factors.

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ACE = angiotensin-converting enzyme
MIF = macrophage migration inhibition
IL = interleukin

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