Direct Immunofluorescence for the Study of Cutaneous Drug Eruptions

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Gibson LE, Van Hale HM, Schroeter AL. Direct immunofluorescence for the study of cutaneous drug eruptions. Acta Derm Venereol (Stockh) 1986; 66: 39–44.

Forty-one patients with drug eruptions were retrospectively evaluated to correlate clinical, direct immunofluorescence, and dermatopathologic findings. Forty-six biopsy specimens were obtained for immunofluorescence in addition to specimens for dermatopathologic study. Eighteen patients had blood vessel fluorescence. Fourteen patients had basement membrane zone fluorescence. Eleven patients had dermatopathologic evidence of lichenoid changes; 8 of these had basement membrane zone fluorescence. Triamterene-hydrochlorothiazide caused the most eruptions (six patients). Positive direct immunofluorescence findings may aid the diagnosis of drug eruptions, especially in the presence of a nonspecific clinical picture or histopathologic findings. *Key words: Dermatopathology; Vasculitis; Lichenoid changes; Triamterene-hydrochlorothiazide.* (Received March 26, 1985.)

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The cutaneous manifestations of drug reactions are protean. Most reactions have a limited nature and course and result in minor morbidity; however, some can be associated with life-threatening systemic reactions. The clinical appearance of many drug eruptions may resemble that of various other dermatoses; therefore, the manifestations of a true drug reaction are often difficult to separate or differentiate from those of an underlying or a concurrent disease. Direct and indirect immunofluorescence studies have previously been used to evaluate drug eruptions; however, the data are sparse and the interpretations are often conflicting (1–7).

To determine whether direct immunofluorescence studies have a role in the evaluation of cutaneous drug eruptions, we retrospectively correlated the clinical findings, dermatopathologic features, and direct immunofluorescence patterns in skin biopsy specimens of patients with drug reactions.

MATERIAL AND METHODS

The results of direct immunofluorescence studies performed on skin biopsy specimens from patients whose final clinical diagnosis was a drug reaction were retrospectively analysed. Between January 1979 and December 1982, 61 such cases were studied in the dermatoimmunopathology laboratory. All patients had been evaluated by a dermatologist, and only those patients with histories and physical findings consistent with a drug eruption were included. A total of 46 biopsy specimens were obtained from the 41 patients included in this study: 42 were from lesional skin, 2 were from perilesional skin, and 2 were from uninvolved skin. The 4-mm or 5-mm punch biopsy specimens were snap frozen in liquid nitrogen and transported to the dermatoimmunopathology laboratory, where frozen sections (4 μm thick) were used for staining with fluorescein-labeled goat antibody to human IgG, IgM, IgA, C3, and fibrinogen. The conjugate concentrations used were: IgG, 25 μg/ml; IgM, 40 μg/ml; IgA, 40 μg/ml; C3, 34.2 μg/ml; and fibrinogen, 16 unitage. Slides were examined by using a Leitz Ortholux II binocular microscope with epi-illumination, and fluorescence was graded for intensity on a scale of 0 (absent) to 3+. All tissue was processed and examined within 48 hours after delivery to the laboratory.

Table I. *Results of immunofluorescence study in 41 patients (46 biopsy specimens) with drug eruptions*

Results	Specimens, no. (%)
Blood vessel fluorescence	18 (44)
Basement membrane fluorescence	14 (34)
Cytoid bodies alone	4 (10)
Intercellular fluorescence	1 (2)
Negative fluorescence	9 (22)

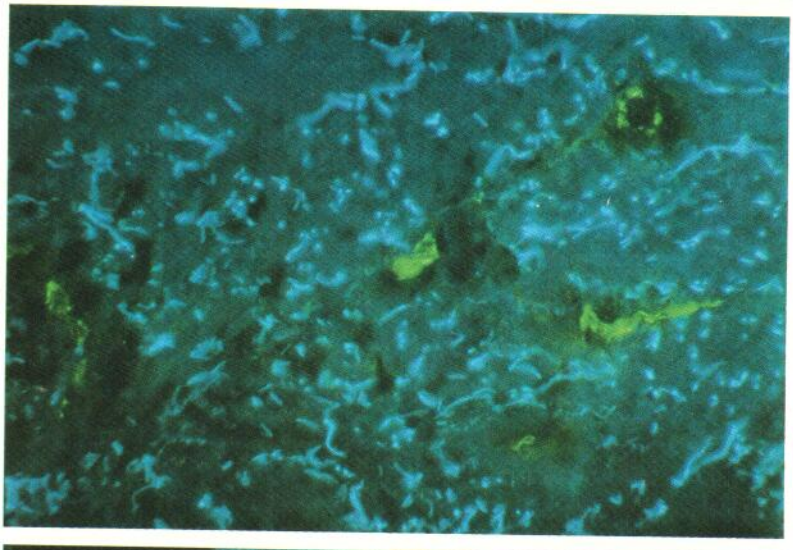
All patients had 4-mm punch biopsy specimens from lesional or perilesional skin for routine histopathologic study. These sections (stained with hematoxylin-eosin) were reviewed and correlated with the corresponding immunofluorescent biopsy.

RESULTS

Blood vessel immunoreactants were noted in the biopsy specimens of 18 of the 41 patients (Table I). In 12 of the 18 specimens, only vessels in the papillary dermis were positive; in 6, only vessels in the reticular dermis or in the panniculus were positive. Fourteen of the 18 specimens had vascular IgM or C3, and 4 had fibrin only. Vascular fluorescence with IgG was not present in any of the 18 positive specimens (Figs. 1 and 2). With light microscopy, only five patients were diagnosed as having necrotizing vasculitis on the basis of invasion of the blood vessel walls by inflammatory cells, endothelial swelling, or nuclear dust; fibrinoid necrosis of vessel walls; and hemorrhage. Four of these five

Table II. *Medications associated with blood vessel and basement membrane zone fluorescence*

Medication	Blood vessel, no. pt	Basement membrane, no. pt
Triamterene-hydrochlorothiazide	2	2
Naproxen	2	1
Indomethacin	–	1
Allopurinol	2	1
Amoxicillin	2	1
Sulfonamide	–	2
Trimethoprim-sulfamethoxazole	2	–
Cardiac antidysrhythmic agents	–	2
D-Penicillamine	1	–
Chlorazepate dipotassium	1	–
Cyclandelate	1	–
Tamoxifen	1	–
Gold sodium thiomalate	–	1
Hydralazine (Apresoline)	1	–
Hydroxychloroquine sulfate	–	1
Prazocin	1	–
Phenytoin	–	1
Ametantrone acetate	1	1
Warfarin sodium	1	–



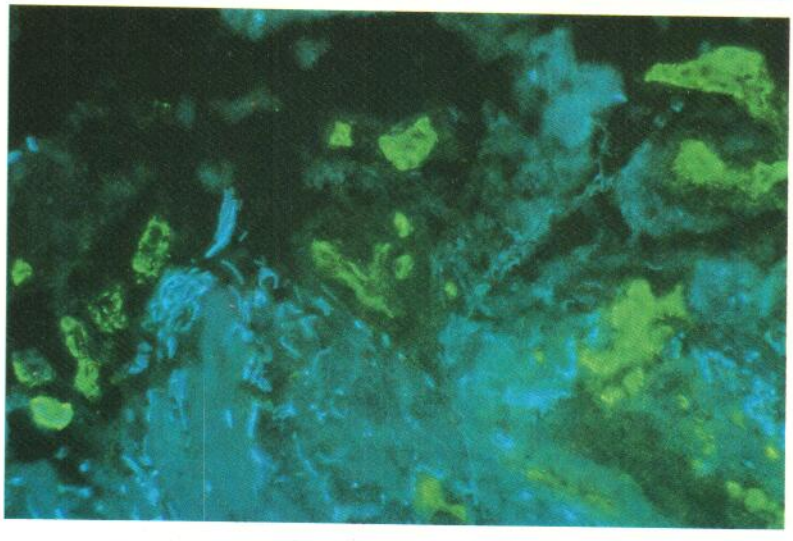


Fig. 1. Fluorescence with IgM in blood vessel of papillary dermis. (Direct immunofluorescence stain; ×550.)

Fig. 2. Fluorescence with C3 in granular basement membrane and blood vessel of papillary dermis. (Direct immunofluorescence stain; ×400.)

patients had blood vessel fluorescence according to the results of direct immunofluorescence: two with involvement in the superficial papillary dermis alone and two with involvement in the reticular dermis.

The drugs implicated in these 18 patients ranged from nonsteroidal anti-inflammatory agents to antibiotics (Table II).

Biopsy specimens of 14 of the 41 patients had basement membrane zone fluorescence. Among these 14 patients, fibrinogen was the only immunoreactant at the basement membrane zone in 8, C3 was found in 4, IgA was found in 1, and IgM was found in 1 (Table III). Dermatologic correlation in the group of 14 patients with positive basement membrane zone fluorescence is summarized in Table IV. The medications implicated in these 14 patients varied (Table II).

Cytoid bodies were frequently found in biopsy specimens of the 41 patients. Of the 20 patients with cytoid bodies, only 4 had them alone, whereas 7 also demonstrated blood vessel fluorescence, 8 had basement membrane zone staining, and 1 had intercellular

Table III. *Immunoreactants in 14 patients with basement membrane zone fluorescence*

Immuno-reactant	Pt, no.	Pattern, no. pt			
		Shaggy	Linear	Granular	Segmental
IgG	0	–	–	–	–
IgM	1	–	1	–	–
IgA	1	–	–	1	–
C3	4	–	1	2	1
Fibrinogen	8	6	2	–	–

substance antibody. In 18 of the 20 patients, cytoid bodies stained with IgM, and 2 of the 20 showed only IgA.

Positive staining in the intercellular space was found in only one patient—an elderly woman with diabetes in whom a vesicular eruption developed after therapy with tolbutamide was begun.

Nine patients with drug eruptions had negative direct immunofluorescence studies. Dermatopathologic examination of their biopsy specimens showed only mild, superficial, perivascular, lymphocytic infiltrates in most cases.

Although the medications implicated in the cutaneous reactions were numerous and varied, several were involved more often than others. Triamterene-hydrochlorothiazide was implicated in six patients. Each of the three patients in whom trimethoprim-sulfamethoxazole was involved had a vesiculobullous component to their skin eruptions. Naproxen was thought to be the causative drug in two patients.

Dermatopathologic evaluation of the 41 patients revealed lichenoid changes in 11 patients. Eosinophils were not prominent in these infiltrates. Eight of the 11 patients had positive basement membrane zone fluorescence with fibrinogen alone (Table III).

DISCUSSION

In this study, a significant number of patients (29 of 41) with cutaneous drug eruptions had positive direct immunofluorescence findings. Blood vessel involvement, mainly with IgM, occurred in 18 cases. In normal skin of healthy persons, positive fluorescence in blood vessels is rare (8). In patients with rheumatoid arthritis, immunoreactants may be found in the blood vessels. Up to 67% of patients with rheumatoid arthritis may have positive blood vessel fluorescence in clinically normal skin, whereas as many as 80% of patients with rheumatoid vasculitis will have blood vessel involvement. In patients with dermatitis

Table IV. *Histopathologic pattern in 14 patients with basement membrane zone fluorescence*

Pattern	Pt, no.
Lichenoid dermatitis	8
Mild dermatitis	2
Vasculitis	1
Subepithelial blister	1
Perivascular lymphocytic infiltrate	1
Nonspecific	1

alone, vascular fluorescence occurs in lesional biopsy specimens of up to 23% of cases (9). The percentage of patients with blood vessel fluorescence in our study (44%) is between the values for patients with dermatitis (23%) and those with true vasculitis (80%) (8).

We believe IgM vessel deposits are significant and probably unrelated to the nonspecific fluorescence that is found in injured skin. The finding of IgM fluorescence in the blood vessels of patients with drug reactions has been reported elsewhere (6, 7). The superficial location of this reactant may be related to the nature of the eruption and to the absence of systemic vasculitis. Only 1 of the 18 patients had a nodular eruption indicative of deeper dermal or pannicular involvement. Many of the drugs listed in Table II have been reported to cause vasculitis; this finding further supports the significance of blood vessel fluorescence in these patients (10, 11). Because postcapillary venules are located in the papillary dermis and these vessels are permeable, the site of most immunoreactants may be explained on this basis (12). Increased vessel permeability may explain blood vessel fluorescence in the absence of dermatopathologic findings of vasculitis.

Of the 14 patients with basement membrane zone fluorescence, 8 had histologic findings of lichenoid dermatitis, and 6 of these had fibrinogen at the basement membrane zone on direct immunofluorescence (either in a shaggy or linear pattern), a finding suggestive of lichen planus; 5 of these 6 also had IgM cytoid bodies. Several of the drugs listed in Table II, including naproxen, hydroxychloroquine sulfate, thiazides, diuretics, and gold (4, 6, 13–16), have been reported to cause lichenoid eruptions. One of our patients who was taking naproxen had clinical, histologic, and direct immunofluorescence findings supportive of a lichen planus-type eruption. A recent report also linked naproxen with this type of eruption (15).

Our findings support those of Watanabe et al. (4); 9 of their 11 patients with lichenoid drug reactions had fibrinogen at the epidermal basement membrane zone, and 7 of these 9 had IgM cytoid bodies. There was good correlation between histologic lichenoid inflammation and the presence of changes consistent with this process on direct immunofluorescence.

Ampicillin or penicillin was thought to cause drug eruptions in four patients; intercellular substance antibody was not found in any of these patients, consistent with the findings in a study by Gatter & Bell (5). However, other investigators, using indirect immunofluorescence techniques, have found antibodies to basal cells in patients who are taking penicillin or other medications (1–3). Nine of our patients had negative direct immunofluorescence studies. A possible explanation would be a delay in obtaining a biopsy specimen for several days after onset of the eruption, because immunoreactants can be cleared from the area even though cellular inflammation is present.

CONCLUSIONS

In summary, cutaneous direct immunofluorescence findings in patients with drug eruptions help support the concept of a probable immunologic operative mechanism. The presence of vasculitis or lichenoid change on routine microscopy correlated well with positive findings on direct immunofluorescence. Positive direct immunofluorescence findings seem to be more sensitive than light microscopy for confirming the presence of a drug eruption. However, in the presence of a nonspecific clinical eruption or nondiagnostic findings with light microscopy, direct immunofluorescence may be an aid to the diagnosis of drug eruptions. Because of the varied drugs, we were unable to identify a specific pattern for a particular drug. A prospective study of specific types of drugs with controls for dermatopathologic and direct immunofluorescence examination may elucidate a definite direct immunofluorescence pattern. With the use of drug-specific (fluorescein-conju-

gated) antibody to identify specific drugs in tissue, it may be possible to develop more specificity with regard to drug eruptions.

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