

Detection in vitro of cellular hypersensitivity to drugs

Ross E. Rocklin, M.D.,* and John R. David, M.D. Boston, Mass.

The in vitro macrophage migration inhibition technique was used to detect the presence of cellular hypersensitivity in patients with suspected drug allergies. Blood lymphocytes from 11 patients (3 serum sickness, 1 allergic vasculitis, 2 nitrofuradantoin syndrome, 1 erythema nodosum, 2 erythema multiforme, 1 polymyalgia rheumatica, 1 granulomatous hepatitis) and 10 control subjects were assayed for the in vitro production of migration inhibitory factor (MIF) in response to penicillin G, sulfisoxazole, and nitrofuradantoin. Lymphocytes from 6 of 11 patients with clinical manifestations of drug hypersensitivity produced MIF in response to the suspected drug but not in response to other drugs. The serum from one patient with serum sickness secondary to penicillin allergy stimulated autologous lymphocytes as well as those of control subjects to incorporate increased amounts of ³H-thymidine, a finding that could be explained by the presence of immune complexes in the patient's serum. Lymphocytes from the control subjects did not produce MIF in response to any of the drugs, suggesting that this technique may be of use in the detection of drug allergies.

Several in vitro methods have been used to aid in the diagnosis of drug allergies. These include the basophil degranulation test,¹ passive hemagglutination technique,² and lymphocyte transformation.³⁻⁵ In many cases, results with the use of these techniques have been inconsistent or difficult to interpret.^{2, 6-8}

An in vitro method being used in our laboratory for the detection of delayed hypersensitivity is the macrophage migration inhibition technique.⁹ We have used this method to measure the production of migration inhibitory factor (MIF) by lymphocytes from patients who had experienced drug reactions during treatment with one or more drugs. In addition, ³H-thymidine incorporation was measured in parallel with MIF production in some cases. The results show that MIF production occurs in response to drugs and that the method may aid in the diagnosis of drug allergies.

MATERIALS AND METHODS

MIF assay

The method for the production of MIF by human lymphocytes has been reported previously.¹⁰ Briefly, heparinized blood is sedimented with dextran, and the lymphocytes

From the Department of Medicine, Robert B. Brigham Hospital, Harvard Medical School. Presented in part at the 27th Annual Meeting of the American Academy of Allergy, February 20 to 24, 1971, Chicago, Ill.

Supported in part by United States Public Health Service Grants AI-7685, RR-05669, AM-12051, AM-05577, and a grant from the John A. Hartford Foundation.

Received for publication May 27, 1971.

Reprint requests: J. R. David, Department of Medicine, Harvard Medical School, Robert B. Brigham Hospital, Parker Hill Ave., Boston, Mass. 02120.

*Recipient of a fellowship from the National Arthritis Foundation.

TABLE I. Diagnosis, suspected drug, age, and sex of patients studied

Diagnosis	Drug	Age	Sex
Serum sickness	Penicillin	7	M
Serum sickness	Penicillin	23	M
Serum sickness	Ampicillin	35	M
Allergic vasculitis	Sulfisoxazole	78	M
Nitrofuradantoin syndrome ¹⁶	Nitrofuradantoin	30	F
Nitrofuradantoin syndrome	Nitrofuradantoin	43	M
Erythema multiforme	Penicillin	60	F
Erythema multiforme	Penicillin	48	F
Granulomatous hepatitis	Sulfisoxazole	54	M
Erythema nodosum	Sulfisoxazole	36	F
Polymyalgia rheumatica	Sulfisoxazole	38	F

are separated from the granulocytes and monocytes using cotton-containing tubes. The lymphocytes are cultured in the absence of antigen or, in the present study, in the presence of 50 μ g per milliliter of penicillin G, sulfisoxazole (Gantrisin), or nitrofuradantoin (Furadantin). The cell-free supernatants from lymphocyte cultures are harvested for 3 days, concentrated 5 $\times$ by lyophilization, and assayed for MIF activity on normal guinea pig macrophages in capillary tubes.⁹ Experiments were carried out which showed that when the original dose of drug used in this study (50 μ g per milliliter) was concentrated 5 $\times$, this amount was not toxic to the migration of normal guinea pig macrophages. Human MIF is routinely produced in the absence of plasma or antibiotics. However, because of the haptenic nature of the drugs under investigation, 0.01 ml. of autologous plasma per milliliter of cell suspension, was added to the lymphocyte cultures as a source of carrier protein. The migration was drawn at 24 hours, the area measured by planimetry, and the per cent migration calculated by the following formula: per cent migration = $X/Y \times 100$.

$$X = \frac{\text{area of migration in the presence of supernatant with drug}}{\text{area of migration in the presence of media with drug}}$$
$$Y = \frac{\text{area of migration in the presence of supernatant without drug}}{\text{area of migration in the presence of media without drug}}$$

Controls for drug toxicity, i.e., medium alone and medium plus each drug, were included in each experiment. These were treated in the same manner as the test supernatants.

Antigen-induced ³H-thymidine incorporation

Lymphocytes were cultured at a concentration of 1.5×10^6 cells per milliliter in either autologous or, in some cases, homologous plasma. The dose of drug used was the same as that used for MIF production (50 μ g per milliliter). Streptokinase-streptodornase (SK-SD) was included in each experiment as a positive control. After 6 days, the cultures were pulsed for 3 hours with 0.5 μ c of ³H-thymidine (specific activity 5 mc. per millimole) and the DNA-labeled material extracted and counted as previously described.¹¹ A stimulation index was calculated from the ratio of counts per minute of stimulated cultures per counts per minute of unstimulated cultures. An index of greater than 3 was considered a positive response.¹¹

Patients

Eleven subjects with clinical histories suggestive of a drug reaction during treatment with one or more drugs were studied. The diagnosis, suspected drug, sex, and age of each patient are given in Table I. All patients were tested within 2 weeks of the onset of their symptoms. Control subjects consisted of 7 individuals who had stopped receiving the drug in question 3 to 24 weeks before blood was drawn or 3 persons who were receiving the drug at the time of testing but were without a history of reaction. Blood was drawn from the latter 3 patients 3 to 4 hours after receiving their last dose of drug.

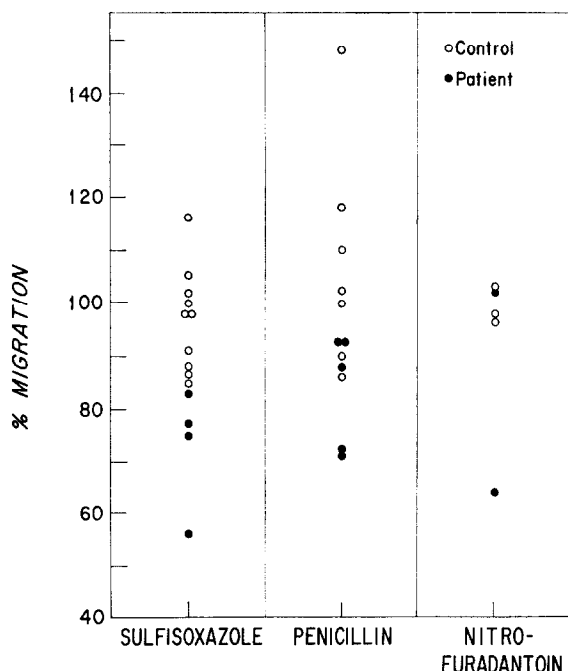


FIG. 1. MIF production by lymphocytes from patients (●) with suspected drug allergies and control subjects (○) in response to sulfisoxazole (Gantrisin), penicillin G, and nitrofuradantoin (Furadantin).

Two of the patients studied were of particular interest, and their case histories will be presented briefly.

Case 1. J. P., a 78-year-old Caucasian man, had a diagnosis at the time of this study of allergic vasculitis. He consulted his physician because of symptoms of a urinary tract infection and was given sulfisoxazole and nitrofuradantoin which he took for 2 weeks. During this time he was also given penicillin. He subsequently entered the hospital with fever and cough. A chest roentgenogram revealed diffuse linear and punctate markings and a pleural effusion. The abnormalities on the roentgenogram gradually disappeared over several months without specific therapy. The white blood cell count and differential revealed a peripheral eosinophilia. All cultures and skin tests for typical and atypical mycobacteria were negative as were stool cultures for ova and parasites. A Kveim test was also negative. A lung biopsy was interpreted as showing a granulomatous arteritis with venulitis and necrosis as well as a giant cell response. Immunofluorescent staining of the biopsy specimen was negative for the presence of IgG and complement. The patient was treated symptomatically and gradually improved over several weeks.

Case 2. D. S., a 23-year-old Caucasian man, developed a serum sickness-type reaction 8 days after he was given penicillin for an upper respiratory infection, despite a previous history of a reaction to this drug characterized by a rash and bronchospasm. Eight days prior to admission he developed fever, chills, and multiarticular joint symptoms. Laboratory studies revealed a normal uric acid, negative latex fixation, negative antinuclear factor, normal antistreptolysin-O titer, and normal serum complement. Cultures of joint fluid were negative, but complement levels were low and a white blood cell count of the fluid revealed 80,000 cells (90 per cent neutrophils). A synovial biopsy showed acute and subacute synovitis. Treatment consisted of supportive therapy and aspirin, and symptoms gradually improved over several weeks.

TABLE II. MIF production by lymphocytes from Case 1 (Patient J. P.) with allergic vasculitis

Patient J. P.	Per cent migration			
	Penicillin	Nitrofuradantoin	Sulfisoxazole	SK-SD
3rd Day hospitalization	102	97	56	73
10th Day hospitalization			76	71
7 Months after hospitalization			60	63

TABLE III. ^3H -thymidine incorporation by lymphocytes from Case 2 (Patient D. S.) with serum sickness and 2 control subjects in autologous and homologous plasma

Subject	^3H -thymidine incorporation (c. p. m.)	
	Autologous plasma background	Homologous plasma background
Patient D. S.	541	50
Control W.	60	176
Patient D. S.	1233	105
Control C.	90	546

RESULTS

The results of the MIF assays in patients and control subjects are illustrated in Fig. 1. None of the control subjects gave a result of less than 80 per cent migration in response to any of the drugs tested. In contrast, lymphocytes from 6 of 11 patients (3 of 4 patients tested with sulfisoxazole, 2 of 5 patients tested with penicillin G, and 1 of 2 patients tested with nitrofuradantoin) gave results below this value. Because of the small number of patients involved in each group, these results cannot be analyzed for statistical significance. However, it should be noted that in previous studies with this technique, migration less than 80 per cent was found to be significant and indicative of MIF production.¹⁰ It is of note that lymphocytes from 3 of the patients who failed to respond to a drug in vitro did respond to SK-SD in the same experiment. Lymphocytes from the other 2 patients who failed to respond to a drug were not tested with SK-SD.

The results of Case 1 are given in Table II. The patient's lymphocytes were cultured with sulfisoxazole, penicillin, and nitrofuradantoin on the third day of hospitalization. It can be seen that of the three drugs only sulfisoxazole elicited MIF production. These tests were repeated, and the results illustrate that the patient's sensitivity to sulfisoxazole could be demonstrated for at least 7 months after he became asymptomatic. SK-SD, an antigen to which he exhibited cutaneous hypersensitivity, was used as a control and was positive on each occasion.

Lymphocytes from the patient with serum sickness (Case 2) were assayed for MIF production in response to penicillin on the second hospital day, and the result of this test was positive (72 per cent migration). Of particular interest was the finding that on two occasions the patient's plasma induced spontaneous incorporation of ^3H -thymidine when it was used in the culture of his

TABLE IV. ^3H -thymidine incorporation by lymphocytes from 6 patients with suspected drug allergies in response to various drugs and SK-SD

Diagnosis	MIF production	^3H -thymidine incorporation (c. p. m.)			
		Penicillin	Sulfisoxazole	Nitrofuradantoin	SK-SD
Serum sickness	Penicillin +	0.9*			4.7
Serum sickness	Penicillin -	0.7	0.7		10.1
Nitrofuradantoin syndrome	Nitrofuradantoin +	1.0	1.0	1.3	15.1
Granulomatous hepatitis	Sulfisoxazole -		0.8		4.6
Polymyalgia rheumatica	Sulfisoxazole +		1.8		7.8
Erythema nodosum	Penicillin -	0.8	0.6		16.7

*Ratio of $\frac{\text{c. p. m. stimulated cultures}}{\text{c. p. m. unstimulated cultures}}$.

cells as well as in those of 2 control subjects (Table III). The patient's cells in autologous plasma incorporated 10 times more ^3H -thymidine than did control cells in autologous plasma, but in the presence of homologous plasma the incorporation of ^3H -thymidine by the patient's cells was normal. Similarly, cells from control subjects incorporated three- to six-fold more ^3H -thymidine when cultured in the presence of the patient's plasma than in their own.

Lymphocytes from 6 other patients were evaluated for drug-induced incorporation of ^3H -thymidine in response to one or more drugs. These results are given in Table IV. It is of note that lymphocytes from these patients were not stimulated by any of the drugs to incorporate significant amounts of ^3H -thymidine, although cells from 3 had produced MIF in response to a drug. Cells from all of the patients had increased ^3H -thymidine incorporation to SK-SD.

DISCUSSION

Use of an assay for delayed hypersensitivity has permitted the detection in vitro of hypersensitivity to drugs. Lymphocytes from patients with a suggestive history of a drug reaction during treatment with one or more drugs were tested for the production of migration inhibitory factor (MIF) in response to the suspected drug(s). The presence of MIF in supernatants obtained from stimulated human lymphocyte cultures was detected by its inhibitory effect on the migration of normal guinea pig macrophages from capillary tubes. The results showed that lymphocytes from 6 of 11 patients (3 of 4 patients tested with sulfisoxazole, 2 of 5 patients tested with penicillin, and 1 of 2 patients tested with nitrofuradantoin) produced MIF in response to these drugs. Lymphocytes from control subjects did not produce MIF in response to any of the drugs. There are several possible explanations for the failure of some patients to respond in vitro: First, although a small amount of serum protein was added to the lymphocyte cultures to act as carrier protein, the drug may not have been immunogenic, especially if some metabolite of the drug was required. Second, the diagnosis of suspected drug reaction may have

been incorrect. Third, a larger dose of drug may have been necessary. Last, there are occasional false negative results with the use of this assay which necessitates repeated testing of individual patients.¹⁰

Employing a different capillary tube technique, Nordqvist and Rorsman¹² demonstrated hypersensitivity to neomycin in patients with eczematous contact dermatitis. This method utilized peripheral leukocytes in capillary tubes in the presence of antigen and 30 per cent autologous plasma. Their results indicated that cells from 7 patients with allergic contact dermatitis were inhibited in the presence of neomycin, whereas cells from control subjects were not.

In the present study, it was of interest that lymphocytes from the patients were not stimulated to increased incorporation of ³H-thymidine when cultured with drugs, although their cells did respond to another antigen (SK-SD). Lymphocytes from 3 of these patients did, however, produce MIF in response to drugs. One reason for the failure of increased ³H-thymidine incorporation in response to drugs may be that larger drug doses are necessary to induce stimulation; however, the dose of drug used was sufficient to elicit MIF production. It should also be pointed out that others have reported difficulty in obtaining increased incorporation of ³H-thymidine in response to drugs in similar patients.^{7, 8} MIF production in the absence of increased ³H-thymidine incorporation in response to an antigen has recently been reported by others.^{13, 14} In the study by Chaparas and associates,¹⁴ it was demonstrated that a tuberculin carbohydrate fraction which could elicit a positive skin test and MIF production did not increase ³H-thymidine incorporation by tuberculin-sensitive lymphocytes in response to this antigen. It is possible that due to their chemical structure, polysaccharides and some drugs are capable only of stimulating certain metabolic pathways in sensitized lymphocytes leading to MIF production but not to proliferation. It is also possible that these antigens stimulate a subpopulation of sensitized lymphocytes which produce MIF but do not stimulate another population to proliferate.

The finding of a factor in the plasma of the patient with serum sickness that induced spontaneous ³H-thymidine incorporation when present in cultures of his cells as well as those of two control subjects was especially interesting. One explanation for this finding might be the presence of antigen-antibody complexes in the patient's plasma. Such spontaneous incorporation of ³H-thymidine by lymphocytes from patients with drug allergies has been described previously.^{3, 15}

The data indicate that the migration inhibition technique may be useful in detection of allergy to drugs, and in addition they demonstrate that drug-induced MIF production can occur in the absence of increased ³H-thymidine incorporation.

The technical assistance of Mrs. Gloria Reardon and Mrs. Carmen Winston is gratefully acknowledged. We are indebted to Drs. Austen, Schur, Bergner, Lovejoy, and Alpert for referring their patients to us to study.

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