

Diagnosis of drug allergy in vitro with the lymphocyte transformation test

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The in vitro test described consists in the transformation of a significant number of small blood lymphocytes into blast cells when lymphocytes from sensitive individuals are cultured in the presence of the specific antigen (lymphocyte transformation test; L.T.T.). Lymphocyte cultures from numerous patients who are sensitive to various drugs (penicillin, aspirin, local anesthetics, various antibiotics, ACTH, insulin, iodinated substances, etc.) have been studied. The test has been regularly positive in the case of clinically and immunologically proved allergy when the antigen was present in the culture. The test was positive whether the allergy was of the immediate or of the delayed type. Corticotherapy or the addition of steroids to the culture medium regularly inhibited the specific lymphoblastic transformation. From the available results it appears that the L.T.T. is a sensitive and reliable test for the diagnosis of drug allergy.

There is no problem in the field of immunology which appears more urgent than the development of a relatively simple and reliable in vitro technique for the diagnosis of drug allergy, actual or potential. Accidents as a result of drug allergies are often serious and may even be fatal. Every year, many deaths caused by the administration of therapeutic or diagnostic agents are reported. Various reports, such as those of the World Health Organization, have emphasized many times that this type of accident is still increasing.

The diagnosis of a drug allergy is sometimes easy and at other times almost impossible to establish. When the clinical history is suggestive and the skin tests are positive, the diagnosis is easily made. This is the case with allergies to heterologous proteins, polypeptides, and, more rarely, to certain drugs. However, in the majority of patients with drug allergies, skin tests are negative in spite of an obvious clinical history. The reasons for these discrepancies are still

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obscure. It may be that most of the drugs are haptens or that the complexes formed are of a special type.

The potential danger of unpredictable and sometimes severe systemic reactions to skin tests in patients allergic to certain drugs, such as penicillin is well known. Therefore, it is very important to have a reliable in vitro technique by which drug allergy may be diagnosed.

The in vitro test based on basophilic degranulation proposed by Shelley¹⁸ has been diversely received. Some investigators have denied any specificity to the test.^{10, 20} Similar opinions have been expressed concerning the passive hemagglutination technique.²¹

It has been shown that human lymphocytes cultivated in vitro in the presence of phytohemagglutinin (PHA)¹⁵ or of specific antigens^{4-6, 13, 14, 17} are transformed into blast cells. We have recently applied lymphoblastic transformation of blood lymphocytes cultured in vitro in the presence of antigen to the study of streptococcal allergy.¹¹ It seemed that lymphocyte transformation might also constitute a sensitive and reliable in vitro technique for the diagnosis of drug allergies; some of the results were reported elsewhere.²²

MATERIALS AND METHODS

Lymphocyte cultures

The isolation and the culture of human circulating lymphocytes were performed according to the technique described by Brandt and associates¹: 60 ml. of venous blood was collected aseptically from each patient. Clotting was prevented by the addition of 0.5 ml. of a calcium heparinate solution containing 5,000 U. of heparin per milliliter. The blood was then filtered through a glass wool column to eliminate the polymorphonuclear cells. The resulting cell suspension contained 40 to 80 per cent mononuclear cells, primarily lymphocytes. The rest of the cells were mostly polymorphonuclear neutrophils which degenerated and disappeared rapidly during culture. The isolated lymphocytes were cultured at a concentration of 1×10^6 mononuclear cells per milliliter in a medium of 30 per cent nonheated autologous plasma and 70 per cent Parker's 199 medium. The medium contained 100 I.U. of penicillin per milliliter, except in those cases where penicillin allergy was suspected, in which case streptomycin (150 mcg. per milliliter) was substituted. All cultures were incubated at 37° C. in test tubes containing 3 ml. of culture. Control cultures were included in each experiment. These consisted of lymphocytes from the same patient which were cultured under the same conditions but in the absence of the drug. The cells were harvested after 4 days of culture. It was found that the percentage of transformation in cultures containing antigen was too low and too hazardous to assess when the culture periods were shorter than 96 hours. Smears were prepared and stained with May-Grünwald-Giemsa stain. A microscopic examination was made of 1,500 cells on each slide and the percentage of transformed cells was recorded. The cells were counted by two different persons, one of them always unaware of the presence or absence of antigens in the culture. Moreover, cultures were done with the lymphocytes of persons whose clinical history was

unknown to the investigator and also of control individuals who were considered normal.

Three types of cells were observed. These included: (a) typical normal lymphocytes (mean diameter, 8 to 10 μ); (b) very large lymphoblast—like cells (mean diameter, 20 to 30 μ) with enlarged nuclei surrounded by a deeply basophilic cytoplasm with numerous vacuoles; and (c) intermediate type cells (mean diameter, 12 to 20 μ). These intermediate type cells were larger than small lymphocytes and smaller than the lymphoblasts but with a distinct lymphoblastic appearance.

Under our experimental conditions the test was considered positive if cultures contained more than 4 per cent blast cells after 4 days of culture in the presence of antigen, provided that the control lymphocyte cultures of the same patients in the absence of antigen and the control cultures of lymphocytes from normal individuals in the presence of the same antigen contained less than 2 per cent blast cells.

Drugs studied

Various drugs were studied; these included polypeptidic hormones, penicillin, aspirin, local anesthetics, iodinated substances, and others.

One of the difficulties encountered was the determination of the optimum concentration of the various substances required to produce a maximum blastogenic effect. Therefore, a preliminary study was necessary to establish the concentration of each substance which produced the highest rate of transformation. For instance, in those studies involving penicillin allergy, it was found that the optimum concentration of penicillin in the culture medium was between 500 and 1,000 I.U. per milliliter. When lower concentrations were used, the percentage of transformed cells was not significant; if higher concentrations (2,000 to 3,000 I.U. per milliliter) were used, then there was a decrease in blast cell production instead of the expected plateau (Fig. 1). When concentrations of penicillin higher than 3,000 I.U. per milliliter were added to cultures, the lymphocytes were damaged. The same phenomenon was observed with some of the other drugs studied, such as streptomycin, procaine, tetanus antitoxin, and iodinated substances.

Some difficulties also arose which were related to the solubility, the pH, and the toxicity of the antigenic substances used. For instance, when acetylsalicylic acid (aspirin) was added to the culture, the optimal concentration of drug in the medium was between 5 and 15 mg. per milliliter. The addition of this drug, even in such small quantities, slightly altered the pH of the medium, and it was necessary to adjust the pH to a normal value with sodium bicarbonate (5.5 per cent), the buffer normally present in Parker 199 culture medium.

Patients studied

A total of 95 allergic patients and 68 nonallergic individuals were studied. They were divided into three groups.

Group I consisted of 30 patients allergic to penicillin, with or without positive

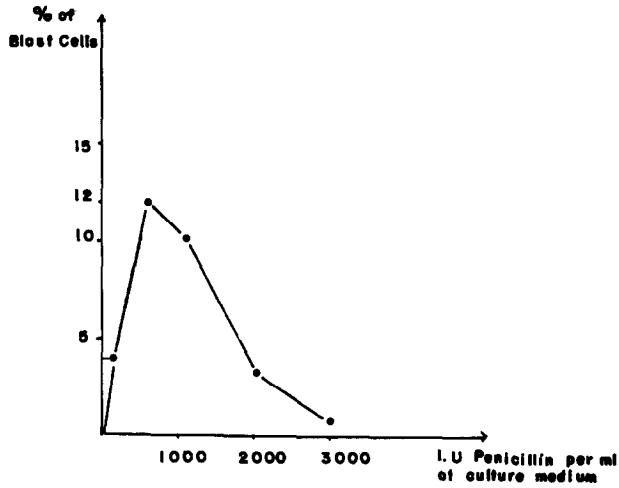


Fig. 1

Variations in the percentage of lymphoblastic transformation as a function of concentration of penicillin in lymphocyte cultures from patients allergic to penicillin. (With concentrations higher than 3,000 I.U. per milliliter cytolysis was observed.)

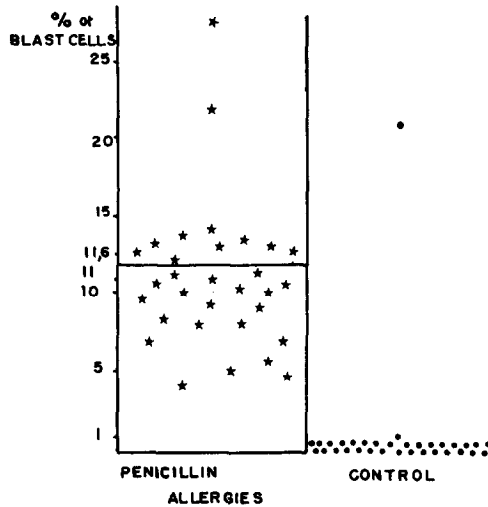


Fig. 2

The horizontal line indicates the mean percentage of transformed cells observed in cultures from patients allergic to penicillin.

skin tests. The 33 control subjects for this group were individuals who had received penicillin and presented no symptoms of penicillin allergy.

Group II consisted of 22 patients with clinical histories of asthma, urticaria, or angioneurotic edema as forms of allergic reactions to aspirin. The 20 control individuals received therapeutic doses of aspirin without developing aspirin allergy.

Group III consisted of 43 patients with clinical and/or immunological evidence of allergy to a variety of drugs. The 15 control subjects had taken various drugs and had no allergic manifestations.

RESULTS

Allergy to penicillin

In this group of 30 patients, 25 presented clinical symptoms of the immediate-type hypersensitivity, such as urticaria, asthma, serum sickness, anaphylactic shock, angioneurotic edema, etc. The other 5 had contact dermatitis. The results obtained from lymphocyte cultures of these patients are shown in Table I. It may be seen that all patients with a history of allergy to penicillin, whether of

Table I. *Lymphoblastic transformation test of patients allergic to penicillin*

Patient No.	Allergic reaction	Culture with antigen		Culture without antigen		Tests*
		Subject (total)	% blast cells	Subject (total)	% blast cells	
A. Patients allergic to penicillin						
1	Rhinitis, asthma		45		0, 4	
2	Asthma, shock, urticaria		22		0, 9	
3	Contact dermatitis		14		0, 1	PT +
4	Contact dermatitis		13, 6		0, 6	
5	Angioneurotic edema, urticaria		13, 1		0, 2	ST + + + +
6	Urticaria		12, 9		0	
7	Urticaria		12, 8		0, 9	
8	Anaphylactic shock		12, 5		0	
9	Anaphylactic shock		12, 5		0, 6	
10	Anaphylactic shock		11, 6		0, 5	
11	Anaphylactic shock		10, 8		0, 3	
12	Anaphylactic shock		10, 7		0	
13	Angioneurotic edema		10, 5		0, 1	ST -
14	Angioneurotic edema		10, 1		0	Shelley -
15	Contact dematitis		10		0, 3	
16	Angioneurotic edema, urticaria		10		0	
17	Asthma, angioneurotic edema		9, 7		0	ST + + + +
18	Asthma, angioneurotic edema		9, 4		0, 5	ST + + + +
19	Contact dermatitis		9, 1		0	IDT +
20	Contact dermatitis		8, 6		0, 1	
21	Shock, asthma, urticaria		8, 5		0, 4	ST +, Shelley -
22	Urticaria, angioneurotic edema		8, 2		0, 4	
23	Urticaria, angioneurotic edema		7		0, 1	
24	Urticaria		5		0, 9	ST -
25	Anaphylactic shock		4, 5		0, 1	
26	Anaphylactic shock		5, 6		0, 5	
27	Anaphylactic shock		7		0, 1	
28	Fever, urticaria		4, 3		0, 2	
29	Anaphylactic shock		11		0, 2	
30	Urticaria		13		0, 8	
B. Controls without penicillin reaction						
Total No., 33						
			15		0	
			16		1	
			1		1	
			1		21, 6	

*Scratch test (ST), patch test (PT), intradermal test (IDT), and Shelley test.

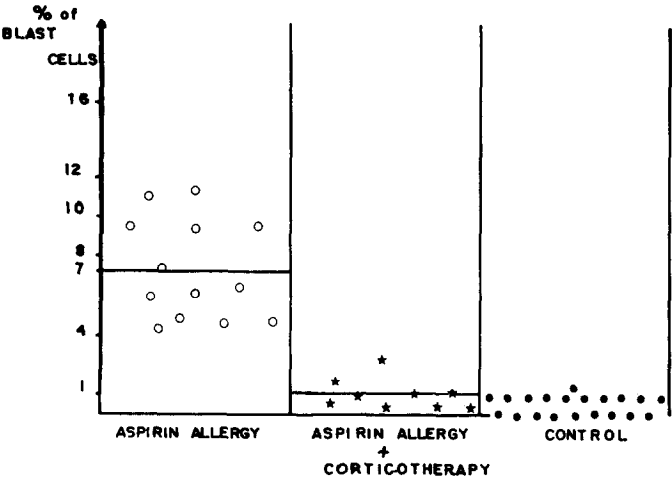


Fig. 3
The horizontal lines indicate the mean percentage of transformed cells in lymphocyte cultures from patients allergic to aspirin.

Table II. *Lymphoblastic transformation test of patients allergic to aspirin*

Patient No.	Allergic reaction	Culture with antigen		Culture without antigen	
		Subjects	% blast cells	Subjects	% blast cells
A. Without corticotherapy					
1	Urticaria		11, 3		1, 9
2	Urticaria, angioneurotic edema		11		0, 3
3	Asthma, nasal polyp		9, 4		0, 5
4	Asthma, nasal polyp		9, 3		0, 3
5	Urticaria, angioneurotic edema		9, 3		0, 1
6	Ocular hemorrhage		7, 3		0, 1
7	Asthma, nasal polyp		6, 4		0
8	Asthma, nasal polyp		6		0, 5
9	Urticaria		5, 7		0, 9
10	Angioneurotic edema		4, 7		0, 3
11	Urticaria		4, 3		0, 4
12	Angioneurotic edema		4, 5		0, 1
13	Urticaria, angioneurotic edema		4, 8		0, 1
B. With intense corticotherapy					
1	Asthma, nasal polyp		3		0, 3
2	Asthma, nasal polyp		1, 8		0, 6
3	Asthma, nasal polyp		1		0, 1
4	Asthma, nasal polyp		1		0, 9
5	Urticaria		0, 8		0, 4
6	Asthma, nasal polyp		0, 4		0
7	Asthma, nasal polyp		0, 4		0, 1
8	Asthma, nasal polyp		0, 6		0, 4
9	Asthma, nasal polyp		0, 4		0
C. Controls without allergic reaction					
Total No., 20			9 : 0	:	:
			10 : 1	:	20 : 0
			1 : 1, 1	:	:

Table III. *Lymphoblastic transformation test of patients allergic to various drugs*

<i>Patient No.</i>	<i>Drug</i>	<i>Allergic reaction</i>	<i>Drug concentration (mcg./ml. or I.U./ml.)</i>	<i>Culture with antigen (% blast cells)</i>	<i>Culture without antigen (% blast cells)</i>	<i>Skin tests*</i>
1	Lignocaine	Collapse	300	7, 6	0, 3	ST (-) IDT (-)
2	Lignocaine	Edema	150	5, 6	0, 1	ST (-)
3	Lignocaine	Eczema	150	7, 5	0, 8	
	Procaine	Eczema	150	4, 5	0, 8	
4	Lignocaine	Anaphylactic shock	150	11, 5	0, 1	PT (-)
5	Procaine	Dermatitis	150	4, 2	0, 2	
6	ACTH	Anaphylactic shock	2,5 I.U.	10, 5	0, 6	
	Synthetic ACTH	None	1,25 I.U.	1, 5	0, 7	
7	Pork insulin	Shock, urticaria	1,5 - 3 I.U.	6, 5	0, 1	
	Ox insulin	Shock, urticaria	1,5 - 3 I.U.	5, 5	0, 2	
	Human insulin	Shock, urticaria	1,5 - 3 I.U.	3, 6	0, 1	
8	Atropine	Urticaria	3 I.U.	6, 2	0, 6	ST ++++
9	Atropine	Dermatitis	15 I.U.	7	0, 1	
10	Gallamine	Shock	150	4, 8	0, 5	ST ++++
11	Gallamine	Shock	150	7, 3	0, 1	ST (-)
12	Iodine	Shock, urticaria	250	10, 9	1, 1	ST (-)
13	Iodine	Angioneurotic edema	250	5	0, 5	
14	Iodine	Urticaria	250	10, 5	0, 1	ST (+)
15	Iodine	Shock, collapse	250	5, 1	0, 2	ST + IDT ++
16	Butyrophenone	Skin rash	3,5	8, 7	0, 6	
17	Spiramycin	Urticaria	30	15	0, 3	ST ++
18	Spiramycin	Erythroderma	30	8, 1	0, 1	
19	Pyrazolone	Urticaria	300	3, 6	0, 1	
20	Tetanus antitoxin	Anaphylactic shock	17 I.U.	8	0, 3	
21	Secobarbital†	Urticaria	35	3, 5	0, 1	
22	Estradiol	Urticaria	35	5, 4	0, 1	
23	Lysozyme	Anaphylactic shock	300	3, 3	0, 1	
24	Riboflavin	Angioneurotic edema	250	3, 6	0, 2	ST +
25	Pyridoxine	Angioneurotic edema	100	3, 7	0, 2	ST +
26	Pentothal	Contact dermatitis	325	4, 6	0, 5	
27	Phenothiazine	Contact dermatitis	17	4, 5	0, 1	
28	Chlorpropamide	Erythroderma	33	5, 2	0, 2	
	Phenylethylbiguanide	Erythroderma	33	5, 8	0, 2	
	Dimethylbiguanide	None	1,000	3, 2	0, 2	

*Scratch test (ST), patch test (PT), and intradermal test (IDT).

†Secobarbital, Seconal, Eli Lilly & Company, Indianapolis, Ind.

Table III—*cont'd.*

Patient No.	Drug	Allergic reaction	Drug concentration (mcg./ml. or I.U./ml.)	Culture with antigen (% blast cells)	Culture without antigen (% blast cells)	Skin tests*
29	Sulfathiazole	Urticaria	33,5	9, 5	0, 1	
	Nicotinamide	Urticaria	33,5	4, 8	0, 1	
	Isoniazid	Urticaria	33,5	3, 4	0, 1	
30	Phenobarbital	Pruritus	150	13, 4	0, 9	ST (-)
31	Phenobarbital	Skin rash	17	12, 6	0, 7	
	Butobarbital	Skin rash	35	9, 6	0, 7	
32	Streptomycin	Angioneurotic edema	150	4, 5	0, 5	ST ±
33	Streptomycin	Contact dermatitis	150	6	0, 4	
34	Streptomycin	Contact dermatitis	150	5, 2	0, 5	PT (-)
35	Streptomycin	Angioneurotic edema	150	8, 1	0, 9	
36	Streptomycin	Erythroderma	150	30	0, 9	PT +++ ST ++
37	Promethazine	Contact dermatitis	9	5, 6	0, 9	PT ++
38	Sulfapyridine	Urticaria	300	10, 3	0, 3	ST ++++
39	Neomycin	Erythroderma	85	9, 9	0, 9	
40	Neomycin	Contact dermatitis	85	4, 4	0, 1	
41	Camposulfonate	Contact dermatitis	3	16	0, 9	
42	Phenylephrine	Contact dermatitis	35	11	0, 1	PT ±
43	Hyoscine	Urticaria	35	10, 3	0, 3	

the immediate or of the delayed type, had a positive L.T.T. The percentage of lymphoblastic transformation varied from 4.3 to 45 per cent (Table I, Fig. 2). The percentage of mitoses observed was small, usually less than 1 per cent. The tests were regularly negative in the absence of antigen. Only one single individual of the 33 control subjects who had previously received penicillin had a strongly positive L.T.T. (see Discussion section).

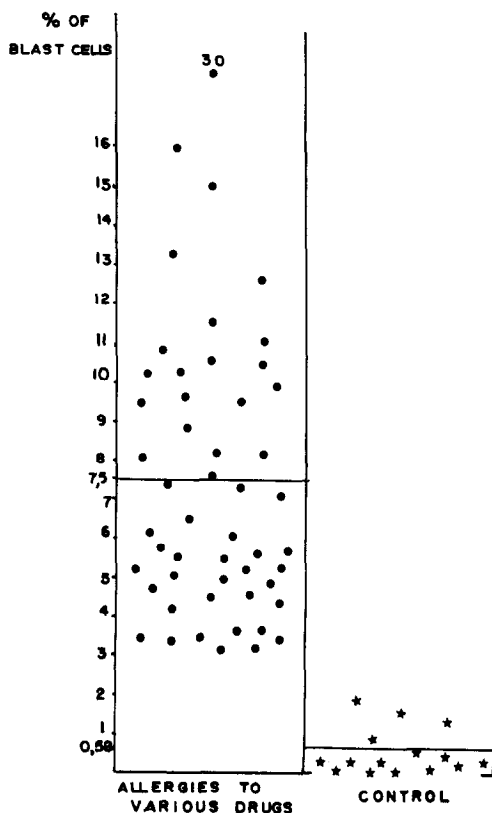
Allergy to aspirin

This group included patients who exhibited asthma, urticaria, and/or angioneurotic edema after ingestion of aspirin.

It was observed that out of the 22 patients with clinical symptoms of aspirin allergy, 13 had a positive L.T.T. The other 9 in whom the test was negative had all received intense or prolonged corticotherapy. The 20 control subjects who had received therapeutic doses of aspirin showed a negative L.T.T. in every case (Table II, Fig. 3).

Allergy to various drugs

The third group of patients consisted of 43 individuals exhibiting clinical and sometimes proved immunological allergic reactions following treatment with

**Fig. 4**

The horizontal line indicates the average percentage of transformed cells in lymphocyte cultures from patients in each group.

various drugs. Since there was a small number of patients allergic to each of these drugs, we included them all in the same table (Table III, Fig. 4).

This third group was very heterogeneous. Although the number of patients allergic to each drug was small, the general impression obtained was the same as that gained from studies of the two preceding groups: the L.T.T. in the presence of the drugs was positive in every case where the clinical history was suggestive of drug allergy.

Conversely, it was consistently negative in the absence of drugs or in the lymphocyte cultures from 15 nonallergic individuals when their lymphocytes were cultivated in the presence of the drugs (Table IV).

DISCUSSION

The results reported here indicate that the lymphocyte transformation test (L.T.T.) in vitro appears to be a reliable technique for the detection and diagnosis of drug allergy. Emphasis is laid on the fact that the number of patients allergic to each drug was often too small to establish reliable statistics.

The test appeared to be positive in both the immediate and the delayed types of hypersensitivity. One advantage of this *in vitro* test is that each subject acts as his own control since the lymphocyte cultures were done in the presence and in the absence of the suspected antigen.

In the group of control subjects who had received penicillin without any clinical symptoms of allergy, only one individual showed a markedly positive L.T.T. (Table I, Fig. 2).

Following this observation, we re-examined the patient. According to the clinical history, he had received treatment with penicillin on two different occasions—the first time in April, 1955, and the second time in April, 1958—and he had shown no clinical signs of allergy whatsoever. The possibility existed, nevertheless, that the sensitization revealed by the lymphocyte transformation test may have occurred as a result of the second treatment with penicillin and remained latent. Therefore, two intradermal injections of penicillin consisting of 5,000 and 10,000 I.U., respectively, were administered into the forearm of the patient; a patch test with penicillin was also performed. The patch test was negative and no immediate reaction was observed at the site of the intradermal injections. However, after 24 hours, an intense inflammatory reaction developed around the site of the penicillin injections; the erythema and edema almost covered the entire forearm. This reaction reached its maximum 48 hours after the injection and persisted for several days.

This patient, therefore, had a typical delayed type of hypersensitivity to penicillin, which is rather uncommon¹⁶ and which, moreover, had remained clinically unapparent. Thus, the *in vitro* test permitted the diagnosis of a potential drug allergy.

The L.T.T. was positive regardless of the chemical nature of the substance. Positive reactions were observed with polypeptidic hormones (ACTH and insulin), with various antibiotics (penicillin, streptomycin, and spiramycin),

Table IV. *Individuals who took various drugs without allergic manifestations*

<i>Patient No.</i>	<i>Drugs</i>	<i>Drug concentration (mcg./ml.)</i>	<i>Culture with antigen (% blast cells)</i>	<i>Culture without antigen (% blast cells)</i>
1	Acetrisoate* and Diodone	250	0, 4	0, 1
2	Gallamine	150	1, 5	0, 1
3	Streptomycin	150	0, 5	0, 5
4	Streptomycin	150	0, 3	0, 2
5	Streptomycin	150	0, 1	0, 1
6	Streptomycin	150	0, 8	0, 3
7	Streptomycin	150	0, 3	0, 1
8	Lignocaine	150	1, 4	0, 1
9	Lignocaine	150	0, 1	0, 1
10	Lignocaine	150	0, 3	0, 2
11	Procaine	150	1, 8	0, 1
12	Procaine	150	0, 1	0, 2
13	Sulfapyridine	300	0	0, 1
14	Pyrazolone	30	0, 5	0, 2
15	Atropine	3	0, 6	0, 3

*Vasurix, Querbet, Paris.

with alkaloids (atropine), and with various other drugs (local anesthetics, iodinated products, and barbiturates). It is important to emphasize the positive reactions observed in lymphocyte cultures in aspirin allergy. Until now, no in vitro or in vivo test could successfully demonstrate allergy to aspirin, the severity and particular significance of which do not need emphasis.

The basic mechanism of the lymphocyte reaction remains a matter of conjecture. The fact that only the lymphocytes of a sensitized individual react in the presence of antigen indicates that these cells carry within them the immunological information which permits them to recognize the antigen. It would be hazardous to speculate on the nature of this information. The fact that the reaction is of the same intensity whatever the type of allergy might be explained in the light of the theory of Karush and Eisen⁹ that delayed hypersensitivity itself is conditioned by circulating antibodies which differ from conventional antibodies only by a particularly high avidity for the antigen. However, the presence of circulating antibodies in this reaction is not evident at all. Another interesting point is that only a fraction of the lymphocytes respond to the antigen. The importance of the fraction varies in a rather wide range. Does this mean that only a part of the lymphocyte population carries the immune information? No answer can be given to this question.

The lymphoblastic reaction appeared to be inhibited in the cultures from individuals receiving steroids (Tables II, B and IV). To prove the effective role of the steroids, in five experiments soluble dexamethasone was added, as well as antigen, to lymphocyte cultures from 5 individuals who were allergic to various drugs (penicillin, streptomycin, phenylephrine, etc.) and who did not receive corticoids. The lymphoblastic transformation was completely inhibited in vitro by the addition of the steroid. In other experiments, patients shown to be allergic to penicillin and to have lymphocytes strongly reactive to penicillin were deliberately given corticosteroids for a week and were then retested. The lymphoblastic transformation test was completely inhibited (Table V). Similar results have since been reported by others.³

Whatever may be the basic explanation of this reaction, its specificity and sensitivity are such that it has been used for the detection of the degree of histocompatibility between two genetically different individuals.⁶ The interest in this test for the detection in man of sensitization to many diverse substances has been emphasized by other investigators.^{4, 6, 11, 13, 14, 17}

Some workers^{7, 19} have shown that lymphoblastic transformation is accom-

Table V. *Percentage of lymphoblastic transformation after corticotherapy in 3 patients allergic to penicillin*

<i>Patient No.</i>	<i>Before corticotherapy (% blast cells)</i>	<i>After corticotherapy (% blast cells)</i>
1	45	2, 6
2	13, 1	0, 7
3	8, 5	1, 1

panied by an increase in the synthesis of DNA, as revealed by the quantity of tritiated thymidine incorporated into the nucleus of the cells.

In our experiments the percentage of mitoses was relatively small. This could be explained by the short duration (4 days) of the lymphocyte cultures. It has already been shown by Holland and Mauer⁷ that the incorporation of thymidine into lymphocytes maintained in vitro occurs principally after the third day of culture under the effect of drugs.

However, the most important cytomorphologic changes observed in the blast cells as a result of antigenic stimulation occurred in the cytoplasm of the cells. They suggested an increased synthesis of cytoplasmic polynucleotides, that is, of RNA and most particularly of ribosomal RNA. This observation agrees well with the demonstration by others of the induction of immunoglobulin synthesis in blast cells stimulated by antigen.

Hulliger and Sorkin⁸ and Landy and co-workers¹² have reported that peripheral lymphocytes synthesize antibody when cultured in vitro. It has also been reported by Girard and colleagues⁴ that fluid from lymphocyte cultures of atopic individuals incubated in the presence of antigen contained an antibody capable of passively sensitizing monkey ileum. These workers demonstrated successfully that significant lymphoblastic transformation occurred regularly in cultures of lymphocytes from patients with immediate-type allergies when allergens were included in the culture medium. They also observed, as we have, that the in vitro lymphoblastic reaction was regularly positive in the case of penicillin allergies when an adequate amount (1,000 I.U.) was added to the culture medium.

Our results and those cited previously are opposed to the negative results reported by Vischer.¹⁹ This author studied the incorporation of tritiated thymidine into lymphocytes of individuals allergic to penicillin and to other drugs. In his study a significant percentage of lymphoblastic transformation was observed in only 2 of 13 individuals allergic to penicillin. No lymphoblastic response was observed in 5 other cases of drug allergy. It is likely that the negative results observed by Vischer were due to the inadequate concentration of the drugs in the medium. This is almost certainly the case with penicillin (Fig. 1) since the author used 100 I.U. penicillin per milliliter of medium in all his experiments. In our hands the optimum concentration of penicillin was found to be 500 to 1,000 I.U. per milliliter of culture medium.

The possibility that the lytic action of high concentration of penicillin which has been observed in our experiments is due to complement activation by soluble complexes formed in excess of antigen cannot be disregarded and deserves verifications.

On the whole, the lymphoblastic transformation test appeared to be a reliable technique for the detection and diagnosis of drug allergy in vitro, but it is also a delicate one. Great care must be taken at every step so that the test is correctly done. The preparation and culture of the lymphocytes must be carried out under conditions of rigorous sterility. The glassware must be very carefully prepared. Perhaps the greatest difficulty is the determination of the optimal quantity of

antigen, and this varied greatly from one substance to another. Some substances are toxic to the lymphocytes. However, when these difficulties were overcome, the L.T.T. seemed to constitute the best test with regard to its precision and reliability in the diagnosis of drug allergy. It is hoped that increased use of the test and many more observations will rapidly confirm our impressions.

CONCLUSIONS

The reaction of lymphoblastic transformation of blood lymphocytes cultivated *in vitro* has been utilized for the detection and diagnosis of drug allergy. Allergies to penicillin, to aspirin, and to many other drugs were studied. The *in vitro* tests have been in agreement with the clinical history and with *in vivo* tests in every case. The reactions have been regularly negative in control cultures in the absence of the antigen. They were also negative in cultures containing antigen when lymphocytes were obtained from nonallergic individuals who had received the same drugs.

The test is positive whether the hypersensitivity reaction is of the immediate or the delayed type. Positive reactions were observed with antibiotics, alkaloids, polypeptidic hormones, local anesthetics, iodinated substances, etc.

It is unnecessary to emphasize the interest of this *in vitro* test for the diagnosis of drug allergy, which is often so difficult and hazardous. However, in order for it to be valid, the test should be performed with extreme care. The determination of the optimal concentration of the active drug to be included in the culture is probably the most troublesome difficulty encountered.

SUMMARY

The development of an *in vitro* technique for the diagnosis of drug allergy is now an urgent problem because of the increasing frequency of severe accidents. The classical techniques such as skin tests and agglutination and precipitation reactions frequently fail because of the nature of either the antigen or the corresponding antibody.

This paper describes a method which, because of its specificity and reproducibility, appears to be very promising. It consists in the transformation of a significant number of small blood lymphocytes into blast cells when lymphocytes from sensitive individuals are cultured in the presence of specific antigen. Lymphocyte cultures from numerous patients sensitized to various drugs (e.g., penicillin, aspirin, local anesthetics, various antibiotics, ACTH, insulin, iodinated substances, etc.) have been studied; appropriate controls were included in each study for comparison. The test has been regularly positive in the case of a clinically proved allergy when the antigen was present in the culture. It was always negative in the absence of antigen or in the case of nonallergic individuals who had taken the same drugs. The test was positive whether the allergy was of the immediate type (asthma, urticaria, anaphylactic shock, etc.) or the delayed type (contact dermatitis). Corticotherapy regularly inhibited lymphoblastic transformation, both *in vivo* and *in vitro*.

Although the test must be done very carefully, it seemed to be a most sensitive and reliable test for the diagnosis of drug allergy.

REFERENCES

1. Brandt, L., Borjeson, J., Norden, A., and Olsson, I.: Separation of Lymphocytes From Peripheral Blood by Means of a Glass Wool Column. A Method for "in Vitro" Culture of Lymphocytes, *Acta med. scandinav.* **172**: 459, 1962.
2. Caron, G. A., Sarkany, T., Williams, H. S., and Todd, A. P.: Radioactive Method for the Measurement of Lymphocyte Transformation "in Vitro," *Lancet* **2**: 1266, 1965.
3. Elves, M. W.: Suppression of Antibody Production by Phytohaemagglutinin, *Nature* **213**: 495, 1967.
4. Girard, J. P., Rose, N. R., Kunz, M. R., Kobayashi, S., and Arbesman, C. E.: "In Vitro" Lymphocyte Transformation in Atopic Patients, Induced by Antigens, *J. ALLERGY* **39**: 65, 1967.
5. Hashem, N., and Barr, M. L.: Mitogenic Effect of Rabies Vaccine on Cultures of Lymphocytes in Diseases of Nervous System, *Lancet* **2**: 1029, 1963.
6. Hirschhorn, K., Bach, F., Kolodny, R. L., Firschein, I. L., and Hashem, N.: Immune Response and Mitosis of Human Peripheral Blood Lymphocytes "in Vitro," *Science* **142**: 1185, 1963.
7. Holland, P., and Mauer, A. L.: Drug-Induced "in Vitro" Stimulation of Peripheral Lymphocytes, *Lancet* **1**: 1368, 1964.
8. Hulliger, L., and Sorkin, E.: Synthesis of Antibodies by Blood Leucocytes of the Rabbit, *Nature* **198**: 299, 1963.
9. Karush, F., and Eisen, H. N.: A Theory of Delayed Hypersensitivity. The Main Features of the Phenomenon are Explicable in Terms of High Affinity Humoral Antibody, *Science* **136**: 1032, 1962.
10. Kravis, L. P., Leeks, H. I., and Whitney, T.: Basophil Degranulation Test in Atopic Allergic States. A Pilot Study of Ragweed Pollen Sensitive Patients, *J. ALLERGY* **36**: 23, 1965.
11. Ky, N. T., Halpern, B. N., Lagrue, G., and Langlois, C.: La transformation lymphoblastique "in vitro" des lymphocytes sanguins appliquée à l'étude de l'allergie microbienne, *Presse méd.* **74**: 1119, 1966.
12. Landy, M., Sanderson, R. P., Bernstein, T. M., and Jackson, A. L.: Antibody Production by Leucocytes in Peripheral Blood, *Nature* **204**: 1320, 1964.
13. Ling, N. R., and Husband, E. M.: Specific and Non-specific Stimulation of Peripheral Lymphocytes, *Lancet* **1**: 363, 1964.
14. Lycette, R. R., and Pearmain, G. E.: Further Observations on Antigen-Induced Mitosis, *Lancet* **2**: 386, 1963.
15. Nowell, P. C.: Phytohemagglutinin: An Initiator of Mitosis in Cultures of Normal Human Leukocytes, *Cancer Res.* **20**: 462, 1960.
16. Redmond, A. P., and Levine, B. B.: Delayed Hypersensitivity in Man, *J. ALLERGY* **37**: 98, 1966.
17. Ripps, C. S., and Fellner, M. J.: Lymphocytes and Drug Hypersensitivity, *Lancet* **2**: 803, 1966.
18. Shelley, W. B.: Indirect Basophil Degranulation Test for Allergy to Penicillin and Other Drugs, *J. A. M. A.* **184**: 171, 1963.
19. Vischer, T. L.: Lymphocyte Cultures in Drug Hypersensitivity. *Lancet* **2**: 467, 1966.
20. Dölen, T., and Aas, K.: Studies of Hypersensitivity to Fish. An Evaluation of the Indirect Basophil Degranulation Test for the "in Vitro" Demonstration of Hypersensitivity to Fish, *Internat. Arch. Allergy* **30**: 405, 1966.
21. Augustin, R.: Precipitins, Reagins and Blocking Antibodies, *Acta allergol.* **16**: 473, 1961.
22. Halpern, B., Ky, N. T., Amache, N., Lagrue, G., and Hazard, J.: Diagnostique de l'allergie médicamenteuse "in vitro" par l'utilisation du test de transformation lymphoblastique (T.T.L.), *Presse méd.* **75**: 461, 1967.