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DIAGNOSIS OF DRUG INDUCED LIVER INJURY USING A LYMPHOCYTE BLAST TRANSFORMATION TEST BASED ON WHOLE BLOOD CULTURE

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Abstract. Twenty cases of suspected drug induced liver injury (16 cases of which satisfied the criteria for at least a query positive diagnosis as based on the Fourth Congress of "Drugs and the Liver" in Japan) were studied by the whole blood culture technique of lymphocyte blast transformation. The positive rate with this technique was 10%, and no more than 15% even with the addition of one query positive. One reason for the low positive rate was that there was not only an allergic mechanism at work in the study group but that liver injury due to direct cytotoxicity of the drug was involved also. For a drug such as chlorpromazine with strong cytotoxicity for lymphocytes, it was difficult to demonstrate a relationship between allergic mechanisms and the drug with this method.

The administration of new drugs and an increased consumption of medicines have resulted, over the past few years, in a substantial increase in the incidence of drug induced liver injury (1, 2). This problem now, therefore, holds an important place in clinical medicine.

Drug induced liver injury can be broadly divided into two groups. One is liver damage due to drug allergy; the other is toxic liver damage in the strict sense. It is thought that the majority of cases are due to some form of allergy to the pharmaceutical involved, but the actual genesis is often unclear in the case of individual drugs.

It is essential to determine the causative drug in the diagnosis of drug induced liver injury, and at present, this is mainly carried out by carefully questioning the patient about previous exposure to drugs. This can be no more than conjecture, however, and the most effective way of characterizing the causative drug is by re-exposing the patient to the drug in question. This procedure may induce dangerous allergic reactions. Skin tests usually are only occasionally positive in the diagnosis of drug induced liver injury (3, 4), so they can not be considered useful diagnostically. As an *in vitro* diagnostic test, the lymphocyte blast transformation test first used in the diagnosis of drug allergy by Holland and Mauer (5) in 1964 has also been applied to the diagnosis of drug induced liver

injury and has proven useful (6-9).

Recently, Mizoguchi *et al.* (10) reported that it was possible to determine the causative drug in drug allergic hepatitis by using a micro-blood culture version of the lymphocyte blast transformation test. In comparison to the traditional technique of using separated lymphocytes, this technique requires only a small amount of blood, so it is very easy to study the nature of the reaction of lymphocytes to various drug concentrations, and to determine the relevant optimal drug concentrations.

The present work, therefore, was undertaken to examine the etiological mechanism of drug induced liver injury as well as an assessment of the usefulness of the whole-blood-culture lymphocyte blast transformation test in the diagnosis of this problem.

MATERIALS AND METHODS

Study group. The study group comprised 20 cases of suspected drug induced liver injury who presented to the First Department of Internal Medicine, Okayama University Hospital or to its affiliated hospitals during the period from August, 1975, to December, 1976 (Table 1). Lymphocyte blast transformation tests were performed by the addition of either the causative drug, or purified protein derivatives of tuberculin (PPD) in a further 2 cases of drug eruption without liver injury and in one normal person with a positive tuberculin reaction.

As is shown in Table 1, 15 (cases 1-15) of the 20 cases studied satisfied the diagnostic criteria 1, 2 or 1, 3 of drug induced liver injury based on the Fourth Congress of "Drugs and the Liver" (Table 2). The remaining 5 cases did not fulfil the criteria but were strongly suspected of being drug induced liver injury from their clinical course. The 28 types of suspected causative drugs in these cases are listed in Table 1 and include 4 cases of ampicillin, 3 cases each of sulphonamide and halothane, and 2 cases of sulpyrin. In 15 of these cases, biopsy specimen obtained by peritoneoscopy was used for histological diagnosis.

The two cases of drug eruption had generalized erythema after the administration of cephazolin or sulpyrin. However, in these cases no liver injury was demonstrated. Case 4, a suspected case of drug induced liver injury, developed generalized erythema due to ampicillin administration after liver function tests had returned to normal. A test of re-exposure to the same drug confirmed its causative role in the drug eruption, so the lymphocyte blast transformation test was performed using this drug.

Methods. The lymphocyte blast transformation test was performed using whole blood culture as in the method of Mizoguchi *et al.* (10) (Table 3). Depending on the number of drugs to be tested, 2-5 ml of blood was taken from the patient and mixed with 10 units of Heparin (Novo Co.) per ml of blood. Aliquots of 0.1 ml were then aseptically placed in small test tubes and 0.01-0.1 ml of test solution together with Eagle MEM added to make a total volume of 1.0 ml.

Controls in which only Eagle MEM was added to the blood were also set up. To each test tube, 200 units of penicillin G and 100 μg of streptomycin were added as antibiotics but this step was omitted where these drugs were possible causative agents.

When the drug to be tested was water soluble, it was added to each test tube in a concentration approximately 1/5000 of the one-shot dose normally given to

TABLE 1. DETAILS OF STUDY GROUP

Patient no.	Age	Sex	Suspected causative drug	Latent period (days)	Fever	Rash	Pruritus	Jaundice	Eosinophils (%)	WBC (No./mm^3)
1	63	M	Dideoxy-KM-B Halothane Cephazolin	10 10 10	−	−	+	+	1	1100
2	52	F	Iopanoic acid Ampicillin	2 2	+	+	+	+	3	2300
3	32	F	Propylthiouracil	76	−	−	+	+	2	3900
4	28	F	Chloramphenicol Cephaloridine Sulfadimethoxine Ampicillin*	30 30 30 4	+	+	+	+	1	7600
5	62	M	Erythromycin estolate	31	−	−	+	+	0.5	3125
6	49	F	Ampicillin	9	−	−	+	+	12	4500
7	31	F	Ampicillin	120	−	+	+	−	3	8200
8	43	F	Halothane	—			+	+		12600
9	52	F	Sulfisomezole	30	−	+	+	+	1	5500
10	27	F	Triacetyloleandomycin + tetracycline	1	−	−	−	−	0	13000
11	55	F	Ajmaline	9	+	+	−	+	0	4900
12	26	F	Ampicillin Acetoaminophen Salicylamide	7 7 7	+	+	−	+		
13	62	M	Streptomycin Isoniazid	59 59	−	−	−	−	16	7900
14	59	M	Pyridinol carbamate Trychlormethiazide	90 97	−	+	+	+	1	9300
15	51	M	Chlorpromazine	36	−	−	+	+		
16	—	M	Sulfisomezole Sulpyrin Indomethacin Aminopyrin	— — — —				+		
17	18	F	Propranolol Clofibrate	2 86	−	−	−	−	2	3500
18	60	M	Hexamethonium bromide Oxytetracycline Sulpyrin	20 14 14	−	−	−	−	1	7500
19	34	F	Halothane	8	+	−	−	−	3	5200
20	23	F	Paraaminosalicylic acid	14	−	−	−	−	1	4350

* Proven cause of a drug eruption in this patient.

TABLE 2. DIAGNOSTIC CRITERIA, HISTOPATHOLOGICAL CLASSIFICATION AND IMMUNOLOGICAL DIAGNOSTIC CRITERIA FOR DRUG-INDUCED LIVER INJURY

Drug induced liver injury can be broadly divided into two groups. One is toxic liver injury, and the other is liver injury due to drug allergy. It is thought that the majority of cases are due to some form of allergy to the pharmaceutical involved. (These criteria are applied for the liver injury due to allergy.)

I. Diagnostic criteria

1. Disturbed liver function one to four weeks* after drug ingestion
2. The appearance of fever, rash, pruritus, and jaundice of skin or mucous membranes as initial symptoms (more than two of these to constitute a positive finding)
3. Eosinophilia (more than 6%) or leucocytosis
4. Liver injury provoked by accidental re-medication
5. A positive lymphocyte culture test

* the period is not strictly limited.

Definite diagnosis requires the presence of 1 and 4, or 1 and 5.

Suspected diagnosis must fulfill 1 and 2, or 1 and 3.

II. Histopathological classification

Hepatocellular type:

In this type, histological changes in the liver resemble those of acute viral hepatitis. Namely, degeneration and necrosis of liver cell constitute the main findings, the area of necrosis being focal, zonal or sometimes massive. Hepatic cells unaffected by necrosis show remarkable variety in size and staining. Cell infiltration and enlarged or proliferated Kupffer cells are present in sinusoids, and infiltration of round cells, neutrophils and sometimes eosinophils together with proliferation of bile ductule are seen in the portal area.

Cholestatic type:

In this type, cholestasis in the central area of the lobule is the most remarkable finding. Bile plugs in the bile capillaries and bilirubin deposits in hepatic cells are dominant. There is little hepatocellular necrosis and cell infiltration of sinusoids is rare. Neither inflammatory reactions nor bile ductule proliferation is ever present. A typical causative drug of this type is C_{17}-alkylated anabolic steroid hormone.

Combined type:

This type has features of both cholestatic and hepatocellular types. Any of the following may be seen: bile plugs in the central area of lobules, bilirubin deposits in liver cells; degeneration and necrosis of liver cells; infiltration of round cells, neutrophils, eosinophils; and bile ductule proliferation.

III. Immunological diagnostic criteria

Lymphocyte transformation test

1. Morphological observtion of blast cells—at least 5% of blast cells to be present for a positive response.
2. Separated lymphocyte culture method
   a. ^{3}H-uridine uptake—at least 170% uptake needed to be positive
   b. ^{3}H-thymidine uptake—at least 200% uptake needed to be positive
3. Whole blood culture method
   a. ^{3}H-thymidine uptake—at least 170% uptake needed to be positive

TABLE 3. PROFORMA FOR THE LYMPHOCYTE BLAST TRANSFORMATION TEST BY WHOLE BLOOD CULTURE

0.1 ml heparinized blood
↓
Mix with 0.9 ml Eagle MEM containing 200 unit penicillin and 100 μg streptomycin
↓
Add an antigen (causative drug etc.) or PHA
↓ Incubate at 37°C with 5% CO_2 and 95% air for 24 hr
Add 1 μCi of ^{3}H-thymidine
↓ Incubate at 37°C with 5% CO_2 and 95% air for 24 hr
Add 3 ml distilled water
↓
Filtrate through a glass filter (GF/C, Whatman)
↓
Wash the filter with 10 ml cold physiological saline
↓
Wash the filter with 5 ml cold physiological saline
↓
Wash the filter with 5 ml cold 5% TCA
↓
Dry the filter
↓
Count by a liquid scintillation counter

an adult. This was based on the fact that, clinically, a normal dose is diluted by the total blood volume in man (5000 ml).

For non-water soluble drugs, fetal calf serum was added to the drug. This was shaken at room temperature for 10 min, centrifuged at 2000 r. p. m., and 0.01-0.1 ml of the supernatant used as the test drug solution. In these cases, a proportionate amount of fetal calf serum was also added to the controls.

With halothane, the concentration was 71 mg/dl by the above method. It was estimated that at the time of addition of drug solutions, the concentration of halothane in the culture fluid ranged from 7.1 up to 71 μg/ml. In case 2, iopanoic acid (Telepaque), which is insoluble in water, was omitted and a similar iodine compound, iodipamide (Biligrafin), used in its place. A further test consisted of adding 0.01 ml of PHA (bacto-phytohemagglutinin-M, Difco Lab., 1 vial dissolved in 5 ml of Eagle MEM) in place of the test drug solution and repeating the experiments using the same protocol.

Culture was performed in a sterile carbon dioxide incubator under an atmosphere of 5% carbon dioxide and 95% air for 48 hr. The small test tubes were inclined at a slope of approximately 15° to the horizontal. Twenty-four hr prior to the completion of culture, 10 μl (1.0 μCi) of ^{3}H-thymidine (specific activity 10.7 Ci/mmole, radioactive concentration 1.0 Ci/ml, Daiichi Chemicals) diluted in 10 times its volume of Eagle MEM was added. After the termination of culture, hemolysis was produced by adding 3 ml of distilled water and the fluid was then immediately transferred to a glass filter (GF/C, diameter 2.4 cm, Whatman) and suction applied. The filter was washed first with 15 ml of cold physiological saline, then with cold 5% trichloracetic acid, dried, 5 ml of "Sintillator" (toluene 1 L, PPO 5.0 g, POPOP 0.1 g) added, and the uptake measured by a liquid scintillation counter (LS-100C, Beckman). A stimulation index (S.I.) was then calculated by the following formula :

$$\text{S.I.} = \frac{\text{cpm of the drug added group}}{\text{cpm of the control group}}$$

All of the above experiments were performed in duplicate and the average S.I. value for each group calculated.

In one group of the patients, patch and intradermal tests were performed in addition to the lymphocyte blast transformation tests. Routine data recorded for all the subjects studied included clinical symptoms, numbers of peripheral eosinophils and leucocytes, serum GOT (SGOT), serum GPT (SGPT), serum alkaline phosphatase (AlP), HBsAg, γ-globulin (γ-gl.), rheumatoid factor (RF), antinuclear factor (ANF), liver histology, and medical and family histories.

RESULTS

Lymphocyte blast transformation tests using whole blood culture. Culture was performed using identical blood from a normal person as non-treated and PHA-treated groups. The results are shown in Table 4. The average value for the non-treated group (mean ± standard deviation, SD) was 315.3 ± 27.94 cpm and the SD/mean ratio was 8.9%. In the PHA-treated group, the mean was 18687.3 ± 1113.7, an SD/mean ratio of 6.0%.

TABLE 4. ^{3}H-THYMIDINE UPTAKE IN LYMPHOCYTES IN 0.1 ML WHOLE BLOOD FROM A NORMAL PERSON

Tube no.	^{3}H-thymidine uptake (cpm/0.1 ml whole blood)	
	Non-treated group	PHA-treated group
1	263	17628
2	276	20735
3	335	19630
4	340	17686
5	332	18019
6	291	17394
7	319	19022
8	321	19384
9	321	
10	355	

The lymphocyte blast transformation test was performed by using blood from a tuberculin positive person and adding 0.1 μg, 1.0 μg, and 10.0 μg of PPD. This method was also used to determine the most suitable culture time (Table 5). The result was that the 1.0 μg PPD added test had a maximum S.I. of 1.80 at 48 hr culture, while the 10.0 μg test had lower S.I. in the same culture time. For the same concentration, the longer the culture time, the larger the S.I. became.

TABLE 5. LYMPHOCYTE BLAST TRANSFORMATION TEST WITH PPD BASED ON CULTURE OF WHOLE BLOOD FROM A TUBERCULIN POSITIVE NORMAL SUBJECT

Culture time (hr)	Lymphocyte stimulation index			
	PPD (μg/ml)			PHA
	0.1	1.0	10.0	
48	1.45	1.80	1.45	37.35
72	2.16	3.28	2.60	71.60
96	2.20	4.19	5.66	87.10
120	4.11	7.89	—	103.30

The 20 patients in the study group were tested with 3 different concentrations of each of the 4 types of drug listed in Table 1. In each case, the drug showing the maximum S.I. and the amount added to achieve that maximum are shown in Table 6. A comparison of these dosages at a standard value of 1/5000 of the normal one-shot dose showed that water-soluble drugs having an added dose larger than the standard were the mixture of triacetyloleandomycin and tetracycline (Sigmamycin) at 3 times the dose and dideoxy-KM-B, ampicillin, and chlorpromazine each at 2.5 times the dose. The other drugs all had amounts equal to, or less than, the standard.

A positive lymphocyte blast transformation test (S.I.$\geqq$1.70) was seen in 2 (patients 1 and 16) of the study group, a positive rate of 10%. Case 4 had a delayed type reaction with the intradermal test using ampicillin and was query-positive by the skin window method, the technique of Yoshida and Take (11), with the same drug. A lymphocyte blast transformation test was performed using ampicillin which had definitely been the causative agent of systemic erythema on a test re-exposure. The S.I. was 1.41. If an S.I. greater than 1.41 is taken as query positive, one more case (case 2) can be added to the series, so that the incidence of positives becomes 15%. Cases 13, 14, 15 and 20 had stimulation indices less than 1.00 and Case 15 in particular had an extremely low S.I. of 0.48. Of the cases of drug eruption, case 21 had an S.I. of 1.78 and a positive lymphocyte blast transformation test and one other case was negative. The PHA addition tests which were performed at the same time in each case had a mean of 43.51 ± 25.46 in contrast to the mean of 32.61 ± 13.41 for the normal population. Case 8 who had a low S.I. of 1.42 for the PHA added test was receiving prednisolone at the time.

Five cases with negative lymphocyte blast transformation tests were tested with both patch tests and intradermal tests. There were no cases with a classical positive reaction, but Case 9 showed a flare-up at the patch site 23 days after the skin test with sulfisomezole.

TABLE 6. RESULTS OF LMPHOCYTE BLAST TRANSFORMATION TESTS AND SKIN TESTS

Patient no.	No. of drugs tested	Drug with max. S. I.	No. of concs. tested	Conc. with max. S. I. (μg/ml)	S. I.	Interval[a] (days)	S. I. of PHA group	Patch test	Intradermal test
1	3	Dideoxy-KM-B	3	50μg/ml	2. 30	290	25. 88		
2	2	Iodipamide	3	500μg/ml	1. 64	22	7. 18		
3	1	Propylthiouracil	3	100μl/ml[c]	1. 25	101	66. 20		
4	3	Chloramphenicol	3	100μg/ml	1. 19	113	80. 02	(−)	(−)
	1	Ampicillin *b*	3	100μg/ml	1. 41	5	13. 51	(−)	(+)[e]
5	1	Erythromycin estolate	4	25μg/ml	1. 18	23	54. 03	(−)	
6	1	Ampicillin	2	500μg/ml	1. 16	75	34. 90		
7	1	Ampicillin	2	500μg/ml	1. 13	180	64. 25		
8	1	Halothane	3	7.1μg/ml	1. 12		1. 42		
9	1	Sulfisomezole	3	100μg/ml	1. 09	30	85. 60	(−)[d]	
10	1	Triacetyloleandomycin + tetracycline	3	300μg/ml	1. 08	39	33. 25		
11	1	Ajmaline	3	10μg/ml	1. 04	20	32. 56	(−)	(−)
12	3	Ampicillin	2	500μg/ml	1. 01	90	60. 60	(−)	
13	2	Streptomycin	3	200μg/ml	0. 84	95	24. 88		
14	2	Pyridinol carbamate	3	100μl/ml[c]	0. 79	278			
15	1	Chlorpromazine	1	25μg/ml	0. 48	43	73. 59		
16	4	Sulfisomezole	3	50μg/ml	1. 70		8. 44		
17	2	Propranolol	3	5μg/ml	1. 27	11	78. 05		
18	3	Hexamethonium bromide	3	10μg/ml	1. 25	45	24. 50		
19	1	Halothane	3	35.5μg/ml	1. 01	61	35. 87		
20	1	Paraaminosalicylic acid	1	100μl/ml[c]	0. 78	119	35. 49		

a The interval between the onset of drug induced liver injury and the time of this test

b Proven cause of a drug eruption in this patient

c μl/ml

d atypical reaction 23 days after the test

e delayed reaction

Table 7. Clinical and laboratory findings

Patient no.	SGOT (unit)	SGPT (unit)	AlP (B. L. u)	HBsAg	γ-gl. (g/dl)	RA test	ANF	Histology of liver	(Interval[a] (days)	Past history	Family history
1	621	231		(−)	2.32	(±)	(−)	Hepatocellular type	(288)	(−)	(−)
2	86	99	20.7	(−)	1.25	(+)		Other type	(34)	RA[c]	(−)
3	1580	1300	6.9	(−)	3.29	(++)	(−)	Hepatocellular type	(94)	Hyperthyroidism	(−)
4	92	55	3.5	(−)	2.96	(++)	(−)	Hepatocellular type	(84)	Drug eruption	(−)
5	126	250	5.1	(−)	1.31	(−)		Combined type	(41)	(−)	RA[c]
6	215	265	19.5[b]	(−)		(−)		Hepatocellular type	(57)	(−)	(−)
7	216	355	25.0	(−)	1.74	(−)	(+)	Other type	(200)	(−)	(−)
8	1970	1680	6.0	(−)							
9	97	94	5.2	(−)	1.44	(−)	(−)	Hepatocellular type	(40)	Asthma	(−)
10	133	107	28.3[b]	(−)	0.80			Hepatocellular type	(41)	(−)	(−)
11	130	350	12.1	(−)	1.30	(−)		Other type	(36)	Drug eruption	
12	230	520	4.6	(−)	0.45			Hepatocellular type	(38)	(−)	(−)
13	116	103								(−)	Lung tbc.
14	640	720	12.4[b]	(−)	1.60	(−)		Hepatocellular type	(60)	(−)	(−)
15	71	111	9.5	(−)	0.87					Lung tbc.	
16				(−)							
17	960	1230	3.0	(−)	1.10					Hyperlipoproteinemia	(−)
18	365	435	7.8	(−)	1.64	(+)		Other type	(38)	(−)	(−)
19	247	480	9.8	(−)	1.07	(±)		Hepatocellular type	(34)	(−)	(−)
20	400	250		(−)	1.56			Hepatocellular type	(212)	Renal tbc.	(−)

a The interval between the onset of drug induced liver injury and biopsy
b AlP in these three patients were measured as K. A. u.
c Rheumatoid arthritis

TABLE 8. SUMMARY OF CLINICAL AND LABOLATORY FINDINGS

	Total cases[a]	Cases of definite or suspected diagnosis[b]	Cases of definite diagnosis[c]
	No. positive cases / No. examined cases	No. positive cases / No. examined cases	No. positive cases / No. examined cases
Sex Male	7/20 (35%)	6/16 (37%)	2/3
Female	13/20 (65%)	10/16 (63%)	1/3
Age 0-19 year	1/19 (5%)	0/15	0/2
20-39	7/19 (37%)	5/15 (33%)	0/2
40-59	7/19 (37%)	7/15 (47%)	1/2
60-	4/19 (21%)	3/15 (20%)	1/2
Latent period 0-30 days	11/17 (64%)	8/14 (58%)	2/2
31-60	3/17 (18%)	3/14 (21%)	0/2
61-	3/17 (18%)	3/14 (21%)	0/2
Fever	5/18 (28%)	4/14 (29%)	1/2
Rash	7/18 (39%)	7/14 (50%)	1/2
Pruritus	11/19 (58%)	11/15 (73%)	2/2
Jaundice	13/20 (65%)	13/16 (81%)	2/2
Eosinophilia	2/16 (13%)	2/12 (17%)	0/2
Leucocytosis	2/17 (12%)	2/13 (15%)	0/2
SGOT, SGPT levels (≧ 500u.)	6/19 (32%)	5/15 (33%)	1/2
A1P level (≧ 9.0 BLu. or 30 KAu.)	5/16 (31%)	4/13 (31%)	1/2
γ-gl. level 0-0.80 g/dl	2/16 (12%)	2/12 (17%)	0/2
0.81-1.75	11/16 (69%)	7/12 (58%)	1/2
1.76<	3/16 (19%)	3/12 (25%)	1/2
RA test (+)	4/12 (33%)	3/10 (30%)	1/2
(±)	2/12 (17%)	1/10 (10%)	1/2
(−)	6/12 (50%)	6/10 (60%)	0/2
ANF (+)	1/ 5 (20%)	1/ 5 (20%)	0/1
Histology of liver Hepatocellular type	10/15 (67%)	8/12 (67%)	1/2
Cholestatic type	0/15	0/12	0/2
Combined type	1/15 (7%)	1/12 (8%)	0/2
Other type	4/15 (27%)	3/12 (25%)	1/2

a. 20 cases composed of 16 cases of definite or suspected diagnosis of drug induced liver injury and 4 cases which did not fulfil the diagnostic criteria but were strongly suspected of being drug induced liver injury from their clinical course

b. 16 cases composed of 3 cases of definite diagnosis and 13 cases of suspected diagnosis

c. 3 cases of definite diagnosis with positive lymphocyte blast transformation test

Clinical symptoms and laboratory results of the study group. The clinical symptoms and laboratory findings of the study group are shown in Tables 1 and 7. The 20 cases comprising the total study group and the 16 cases who fulfilled the diagnostic criteria in Table 2 for more than a suspected diagnosis are summarized in Table 8. As the total number of cases of definite diagnosis with positive lymphocyte transformation tests (including 1 query positive) was small, only the number of cases is recorded.

There was a sex preponderance of females (10 cases, 63%) in the 16 cases (Cases 1-16) who fulfilled the diagnostic criteria of at least a suspected diagnosis. The latent period for 8 of 14 cases was less than 30 days (58%) and, for the majority of cases, less than 60 days (11 of 14 cases, 79%). The most frequent clinical symptom in the drug induced liver injury was jaundice (13 of 16 cases, 81%), followed by pruritus (11 of 15 cases, 73%), rashes (7 of 14 cases, 50%) and fever (4 of 14 cases, 29%). Eosinophilia occurred in 2 of 12 cases (17%) and leucocytosis in 2 of 13 cases (15%). SGOT and SGPT levels greater than 500 units occurred in 5 of 15 cases (33%). AlP levels exceeded 30 KA units or 9.0 BL units in 4 of 13 cases (31%). All cases were HBs antigen negative. Normal γ-globulin levels (taken as 0.81-1.75 g/dl (12)) occurred in 7 of 12 cases (58%) and the combined number of those exceeding normal values (3 of 12 cases, 25%) and those less than the normal values (2 of 12 cases, 17%), that is, those with abnormal values, totalled 42%.

RF measured by the RA test (Hyland Co.) was positive in 3 of 10 cases (30%), and if those showing (±) reactions were also included, totalled 4 of 10 cases (40%). ANF was positive in 1 of 5 cases examined. Liver histology of biopsy specimens obtained by peritoneoscopy 34 to 288 days after the onset of the syndrome was classified according to the criteria in Table 2. The hepatocellular type was most frequent (8 of 12 cases, 67%), there were no cases of the pure cholestatic type, and there was 1 case (of 12 cases) of the combined type. Further 3 cases which did not comply with this classification were grouped together. These included one case of non-specific reactive hepatitis, one case of plasma cell infiltration in the interstitial tissue, and one case of proliferated bile ductules but in which there was little change in the hepatic cells. A futher one case only (Case 6) showed eosinophil infiltration of the hepatic tissue.

A past history of chronic rheumatoid arthritis, asthma, or drug eruption was presented in 4 of 14 cases (29%). There were no particular family histories.

The above clinical findings did not indicate any large differences among the total group of 20 patients. The finding, however, that the RA test was always at least (±) in the cases which had positive or query-positive lymphocyte blast transformationt tests was characteristic.

DISCUSSION

It is now known that the phenomenon discussed by Nowell (13) in 1964 of lymphocyte blast transformation during culture with PHA added to the medium also occurs during the culture of peripheral lymphocytes with specific antigens added (14, 15). In drug allergy, it is generally not possible for the administered drug, by itself, to become an antigen but it is considered that, working as a hapten and binding with the individual's proteins, that is, carrier proteins, the drug acquires antigenicity and induces an allergic reaction (16, 17). In the study of patients with drug allergies, Holland and Mauer (5) were first to use *in vitro* addition of the suspected drug as a hapten, perform lymphocyte culture, and, by observing the lymphocyte blast transformation phenomenon which occurred, characterise the causative drug. Various workers are continuing this research (18-21).

In drug induced liver injury it is generally thought that the drug combines with protein originating in the liver, acquires antigenicity, and *via* the individual's immunological response induces an allergic response in the liver. There are as yet, however, few reports analysing causative drugs to drug induced liver injury by this method and the positive incidences reported are different from each other (6, 22-25). In Japan, there have been publications by Tsuji *et al.* (7), Namihisa *et al.* (8), Kato (9), Mizoguchi (26), and Yamada (27), and of these, Yamada detected a high rate of positive lymphocyte blast transformation tests (57 of 83 cases) by using a whole blood culture technique.

The present research was an analysis of drug allergy in terms of doses involved and incidence rates in patients who were suspected of drug induced liver injury on clinical examination and liver biopsy, especially patients in whom allergic mechanisms, as defined by criteria based on the Fourth Congress of "Drugs and the Liver", were thought to be involved. The result of whole blood culture was a positive rate of 10% (2 of 20 cases), and even if one query positive case is included, the rate was still only 15%. The author's experimental method had no problems in term of culture conditions and technique. This was indicated by the following ; the standard deviations of cpm of ^{3}H-thymidine uptake in the non-treated and PHA-treated groups for the normal person were small, there was a good response to the addition of PPD in tuberculin positive case, a positive result was obtained in the one case of drug eruption thought to be a classical drug allergy, and there was an adequate response of the S.I. in all cases (excluding case 8 undergoing prednisolone treatment) to the addition of PHA.

Furthermore, there was no great difference between the present experiments and those of other workers in either concentration of drugs used or intervals between the onset of the syndrome and the time of this test. The reason for the low

incidence of positives in spite of selecting patients who were strongly indicated clinically as having allergy type drug induced liver injury can be considered as being due to the inclusion of cases in whom a positive result for the lymphocyte blast transformation test was difficult to obtain. Dove *et al.* (23) suggested that the absence of positive lymphocyte blast transformation tests in patient with isoniazid-associated hepatitis was possibly related to isoniazid toxicity and this idea is also applicable to the present author's results as well. Especially in the case of chlorpromazine in patient 15, the addition dose was low in comparison with other drugs and certainly was not excessive in terms of usual doses of chlorpromazine, so the demonstration of an extremely low S.I. must be considered as evidence of strong cytotoxicity for lymphocytes. This cytotoxicity can also affect all cells of the body, including hepatic cells, and it is a point of great interest that the cytotoxicity of chlorpromazine, which is a representative drug causing allergic drug induced liver injury, may be associated with the mechanism of drug induced liver injury. Moreover, this technique was considered unsuitable for the investigation of allergic mechanisms in the case of drugs having such marked cytotoxicity for lymphocytes. At present, the macrophage migration inhibition test is also used in diagnosis of allergy type of drug induced liver injury, and in this method the cytotoxicity of drugs is a problem because of the inhibition due to the cytotoxicity of added drugs.

On the other hand, concerning the cytotoxicity itself, it may be thought that this whole blood culture method has possibilities as a method to study drug cytotoxicity *in vitro* for its peculiarity of being able to examine various drug concentrations.

In addition to the problem of cytotoxicity, further problems which influence the positive rate of this experiment remain to be clarified in the future. These problems include the standard for the positive response in this method, the most suitable length of culture time, and the problem of carrier proteins.

Skin tests are a simple technique for the diagnosis of causative drugs in drug induced liver injury, but the positive rate is generally low. Goto *et al.* (28), however, performed patch and intradermal tests in cases of drug induced liver injury in which rashes were also present and diagnosed positive delayed type reactions in all 7 cases. In the author's 5 cases of negative lymphocyte blast transformation tests, all skin test results were regarded as negative, but in one of the cases which had a concomitant rash, a flare-up was seen in the patch area on the 23rd day after exposure to the drug suspected as being the causative drug of the liver injury. The mechanism of this phenomenon is unclear but it is an interesting finding, and in combination with the results of Goto *et al.* (28), it seems partially useful to perform skin tests in drug induced liver injury, especially with rashes. There are no reports of using the skin window method to diagnose the causative

drug in drug induced liver injury, but skin window assessment of the status of eosinophils and basophils, a technique which differs from conventional diagnostic methods, needs further study.

In the 16 cases of the study group which fulfilled the diagnostic criteria, females were in the majority and the latent period was less than two months in the majority. This agrees with various other authors' findings. Clinical symptoms are also included in the diagnostic criteria and as a whole had a high incidence in the group, but fever had a lower rate than other workers' report. This was probably due to the fact that it is more difficult to be certain subjectively about fever than it is for the other symptoms. The incidence of both eosinophilia and leucocytosis in peripheral blood was low and may have been due to the fact that in the most of the cases blood analyses were not performed immediately after the onset of symptoms. Cases with SGOT and SGPT levels greater than 500 units or cases with AlP levels greater than 30 KA units or 9.0 BL units were seen at the same frequency; that is, approximately 1/3 of the total cases. The former was considered to indicate the hepatocellular type clinically, while the latter the cholestatic type. Those cases showing abnomal levels of γ-gl. and those showing positive RA test (including $(\pm)$ reactions) were approximately 40%, and these abnormalities often continued after the other liver function tests returned to normal. This was considered as indicating one aspect of abnormal immunological responsiveness of the individual with drug induced liver injury. The positive ANF case and the family histories of allergy may also reflect this abnormal immunological responsiveness.

Liver histology was represented mainly by the hepatocellular type, there being no cases of the cholestatic type. The four cases grouped as cholestatic type on the grounds of laboratory values were classified histologically as hepatocellular type or types other than the above two. This may have been due to the fact that the liver biopsies were taken more than 34 days after the onset of symptoms when improvement in the various laboratory values had been recognized. It is thought that recovery from cholestatic type of liver injury is relatively prompt and without residual abnormalities. The low incidence of eosinophil infiltration in hepatic tissue was also thought to be due to the time of biopsy.

From all this, it was concluded that, when classifying drug induced liver injury using laboratory data and hepatic histology, it is important to consider the stage at which they were performed in order to accurately interpret the information.

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