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INCREASED SENSITIVITY OF MICROLYMPHOCYTOTOXICITY TEST BY USING THROMBIN - SUCROSE TREATED LYMPHOCYTES

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ABSTRACT

A technique of thrombin treated lymphocytes separated by Ficoll-Hypaque-Sucrose (FHS) technique for microlymphocytotoxicity test is described. It is revealed in this study that 58 (33.14%) and 27 (15.42%) of 175 pregnant women were positive for the anti-HLA screening test by using thrombin treated lymphocytes and untreated lymphocytes, respectively. The enhancing effect of antigen-antibody and complement activity of lymphocytes separated by FHS technique with thrombin treated over those separated by NTTRL and Batchelor method is probably due to both the protease activity of thrombin on the lymphocyte membrane and the high osmolarity of sucrose solution of which during elimination of platelets from the lymphocyte - platelet mixture may cause the sublytic damage of lymphocyte membrane leading to the leak of cation from cells and resulting in the increasing of the sensitivity of lymphocytotoxicity test.

INTRODUCTION

It has been well known that the HLA-SD antigens, were almost recognized through the lymphocytotoxicity test. A weakness of lymphocytotoxin developing in the parous women usually creates the discrepancy and error of the test. Enhancing methods of lymphocytotoxic reaction utilizing frozen cells(1), synergistic effect of sublytic dose of lymphocytotoxic antisera (2), lymphocyte treated with proteolytic enzyme, ficin and

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trypsin(3-5) have been reported. The present method is to describe the effect of lymphocyte treated with thrombin in increasing the sensitivity of a micro-droplet lymphocytotoxicity test.

MATERIALS AND METHODS

Ethylene diamine tetraacetic acid (EDTA) blood (0.1 ml of 5% EDTA per ml of whole blood) was obtained from blood donors and parous women who attended at the Department of Pathology, Ramathibodi Hospital.

The lymphocytes was prepared by FHS method (6)and NTTRL and Batchelor's method.(7)

The microlymphocytotoxicity test (7) was used for comparative study of anti-HLA screening by using thrombin treated and untreated lymphocytes separated by FHS techniques.

A total of 175 pregnant women was compared for HLA typing. Each sera was desingned to test against 20 random and untyped donor's lymphocytes. If more than 30 percent of killed lymphocytes present the reaction is considered positive.

THE RECOMMENDED METHOD FOR THROMBIN TREATED LYMPHOCYTES :

One volume, 0.1 ml, of lymphocyte suspension prepared by FHS method was incubated with an equal volume of working thrombin solution (Thrombin, Topical, Bovine origin, 5000 NIH units, Parke Davis&Co., Detroit, was diluted 1 : 2 with normal saline solution) at 37°C for 15 minutes. After incubation the treated cells were washed with Complement Fixation Test (CFT) diluent (CFT; pH 7.4; Barbitone 0.58 gm, sodium chloride 8.5 gm, Barbitone sodium 0.19 gm, $MgCl_2.6H_2O$ 0.17gm, $CaCl_2.2H_2O$ 0.03 gm and 1000 ml distilled water) and centrifuged at 400 G for 2 minutes. The cells were then adjusted to 1000 cells per cu.mm. in CFT diluent for a microcytotoxicity test.

RESULTS

The score of lymphocytotoxic reaction which the lymphocyte suspension was prepared by FHS method, was higher than NTTRL and Batchelor's method and thrombin treated lymphocytes gave higher score than untreated cells and was reduced along with the dilution of thrombin (Figure 1)

The enhanced microcytotoxicity test of lymphocytes treated with thrombin was good in reproducibility. The coefficient of variation (C.V.) was in the range of 3.99 to 8.86% (Mean 6.60) (Table 1) which was below the acceptable limit (C.V. 15%).

In 175 parous women, lymphocytotoxic antibody was found in 27 (15.42%) and 58 (33.14%) using thrombin untreated and treated lymphocytes, respectively.

DISCUSSION

The sensitivity of the present microcytotoxicity test might be the effect of lymphocyte membrane damage due to the leak of cation from the cells during the preparation of lymphocyte suspension through the sublytic dose of the high osmolarity of sucrose solution in the platelet elimination process 6). Another effect was probably due to the property of a trypsin-like proteolytic activity of thrombin(8-10) on the lymphocyte membrane.

The presence of preformed cytotoxic antibodies in a serum of recipient almost causes immediate rejection (11). Furthermore, it was recently known that immediate allograft failure was more frequent in recipients with preformed antibodies(12). Strong preformed antibody would show positive cytotoxicity in a direct cross-match of the serum of recipient with lymphocytes of donor(13). However, negative cross-match might result from the presence of weak preformed antibody. The present method is unsophisticated technique and can eliminate this problem.

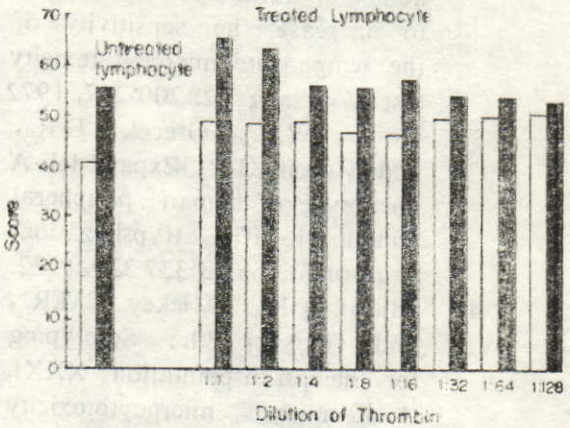

Figure 1 The reactivity score of microlymphocytotoxicity test using thrombin untreated and treated lymphocytes separated by Ficoll and Batchelor's technique □ , and Ficoll-Hypaque-Sucrose technique ▨ . (Mean of 10 experiments).

Table 1 The Score of Reproducibility of Thrombin-Treated Lymphocytes Microcytotoxicity Test.

Experiment	Thrombin-treated lymphocytes										$\bar{X} \pm$ S.D.	C.V.(%)
	1	2	3	4	5	6	7	8	9	10		
I	47	51	58	62	54	53	59	48	52	53	53.70 ± 4.76	8.86
II	60	62	59	63	68	63	63	65	64	52	62.90 ± 2.51	3.99
III	39	39	36	45	42	44	42	42	36	40	40.50 ± 3.06	7.56
IV	58	51	49	51	58	51	54	53	53	57	53.50 ± 3.20	5.99
Mean of C.V.												6.60

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ย่อเรื่องที่ผ่านมาแล้วข้างต้น

การทำ microlymphocytotoxicity test โดยการเตรียม lymphocytes ด้วยวิธี Ficoll - Hypaque - Sucrose ได้หา anti-HLA ในคนตั้งครรภ์ 175 คน ผลปรากฏว่าจะให้ผลบวก 58 (33.14%) คน และ 27 (25.42%) คน ด้วยการใช้ thrombin treated lymphocytes และ non treated lymphocytes ตามลำดับ ปฏิกิริยาที่ดีขึ้นอาจจะเป็นผลจาก protease activity ของ thrombin และ high osmolarity ของ sucrose ต่อ membrane ของ lymphocytes ในระหว่างการเตรียม lymphocyte suspension จึงทำให้ผลของการหา anti-HLA ในคนตั้งครรภ์ดีขึ้นดังกล่าว.