Dedection of Cell Mediated Immunity To Entamoeba Histolytica By Leukocyte Adherence Inhibition Test

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AMOEBİASıS LOKOSİT ADHERANS tNHİBİSYON TESTİ

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SUMMARY

In this research we have applied LAI test in order to determine cellular immune response to amoebic infection. For this purpose, a soluble antigen was prepared from E. histolytica and in vitro immune response were observed in persons with amoebiasis. and the results were compared with controls.

Fifteen patients with amoebiasis and fifteen healthy controls have beenstudied. LAI test has been evaluated in two groups by counting the adherent cells before and after washing the slides, and the difference between the responce of two gloops to antigen was noted. There was no difference between the leukocyte counts in control groups, but the difference between the test groups with and without antigen was statistically significant. It was demonstrated that the response to amoebic antigen differs significantly. The patients showed a cellular immune response to the antigen.

We suggest that there is a cell mediated immune response in amoebiasis and LAI test is a simple and useful-test for this purpose.

Key Words: Entamoeba histolytica, leukocyte adherence inhibition

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ÖZET

Bu araştırmada amoebic infeksiyona karşı hücresel immün cevabı incelemek amacıyla LAI testini uyguladık. Bunun için E. histolytica'dan solubl bir antijen hazırlanmış ve amoebiasisli hastalarda in vitro immün cevap gözlenerek sonuçlar kontrol ile karşılaştmlmıştır.

Amoebiasis'U 15 hasta ve sağlıkh 15 kontrol incelenmiştir. LAI testi, lamların yıkanmasından önce ve sonra yapışmış olan hücrelerin sayımı ile iki grupta değerlendirilmiş, antijeni! ve antijensiz sayımlar arasındaki farklılık kaydedilmiştir. Kontrol grubunda lökosit saytmlart arasında fark bulunmamış, fakat test grubunda antijenli ve antijensiz sayımlar arasındaki fark istatistiksel olarak anlamlı bulunmuştur. Bu durum amoebic antijene karşı cevabın anlamlı derecede farklı olduğunu gösterir.

Amoebiasis'de hücresel bir immün cevabın olduğu ve LAI testin bunu gösterebüen basit ve yararlı bir test olduğu kanısındayız.

Anahtar Kelimeler: Entamocba hislolytica, leukocyte adhercncc ıılıhıbnıon

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INTRODUCTION

Entomoeba histolytica induces not only humoral but also cellular immune response (9, 10). Humoral immune response is characterized by the rapid appearance of circulating antiamoebic antibodies. Most studies are concerned with antigens detectable by serologic methods. Whereas, little work has been done on antigens eliciting cellular immune response (10). For this reason we have investigated cell mediated immunity to E. histolytica. In attempt to clarify the above situation we have applied Leukocyte Adherence Inhibition Test (LAIT).

LAI assay was first described by Halliday and Miller in 1972 (3). This method has been successfully used for monitoring cellular immunity in animals and human (2, 4, 5, 6, 8). It depends upon decrease of glass-adherent property of leukocytes after incubation with specific antigens (3,4).

EMOP IV (The Fourth European Multicolloquim of Parasitology) 14-19 Ekim 1984, İzmir'de ingilizce olarak tebliğ edilmiştir.

This is the first report about the application of LAI assay in amoebiasis.

MATERIALS AND METHODS

15 patients with intestinal amoebiasis and 15 healthy controls were investigated. 10 female and 5 male patients with the history of intestinal complaints were examined parasitologically. All of them were found infected with E. histolytica. Healthy controls were 8 female and 7 male harboring no parasites.

Antigen for LAI assay: Stool samples with E. histolytica filtered through one layer of wet cheese cloth and cultivated in Jones's medium for 7 days at 37 C.

Jones's medium contains the following ingradients:

1. **Buffered saline solution (pll 7.2)**

   Na_2P0_4 (9.48 per litter). . . . .375.0 ml
   K_2HP0_4 (9.08 per litter). . . . .125.0 ml
   NaCl (0.9%). . . . . .2250.0 ml

2. **Basic medium**

   Yeast extract (autolyzed) 1% . . . 100.0 ml
   Saline solution (pH 7.2). . . . . .850.0 ml
   Equine blood serum (sterile). . . . .50.0 ml
   Rice starch (sterile). . . . . .3.0 g

The supernatant was removed after seven days and the sediment collected in a centrifuge tube. It was centrifuged at 500 rpm for 10 min. and then the supernatant was removed again.

The sediment washed with saline solution for three times and was homogenized by freezing and dissolving.

Source of Leukocytes: Blood was collected by venipuncture and were mixed with approximately 20 units of heparin/ml disposable injector. Heparinized peripheral blood (20 ml) was mixed 5:1 with 0.6% dextran (Macrodex) and incubated for 20 min. at 37°C. The leukoycte-rich plasma was centrifuged at 400 x G for 10 min. and sediment was suspended in medium 199 lOx and fetal calf serum to a concentration 1 x 10^7 cell/ml.

Control: 0.1 ml cell suspension, 0.05 ml medium, 0.05 ml normal human serum (control serum).

Test: 0.1 ml cell suspension, 0.05 ml antigen, 0.05 ml normal human serum.

The mixture was incubated in capped plastic tubes in 37 C water bath for 30 min by gently shaking every 5 minutes. They were than transferred on Thoma slide and counted. After an incubation of an hour in humid atmosphere, the coverslip was then floated of the slide by slowly immersing in a 15 cm. Petri Dish was filled with cold Hanks solution. The slide was removed from the dish and held vertically while being immersed in a 100 ml beaker of Hanks solution. After washing, a drop of medium was placed on the slide and a clean coverslip put into place. The remaining adherent cells were counted in the same square as before. Paired t-test was used to obtain the degree of the significance of difference between means.

RESULTS

15 patients with amoebiasis and 15 healthy controls have been studied. The leukocyte counts of two groups before and after washing with and without antigen mixture are shown in Table-1. There was no difference between two groups before washing.

Table - I

LAI Results of Patients and Healthy Controls

	PATIENTS				CONTROLS			
	Before Washing		After Washing		Before Washing		After Washing	
Subjects	A I	B I	A II	B II	A I	B I	A II	B II
1	55	62	40	22	72	58	37	29
2	50	44	36	17	60	55	30	34
3	70	75	52	24	64	60	32	33
4	84	85	60	32	54	67	29	53
5	49	69	31	30	46	62	25	50
6	73	72	58	10	58	73	45	54
7	68	58	50	14	70	54	58	38
8	48	65	39	18	63	69	39	45
9	67	60	50	20	47	66	50	44
10	40	72	32	17	65	55	52	39
11	77	48	51	13	73	75	60	58
12	46	55	30	11	66	70	38	62
13	54	69	42	22	71	74	46	56
14	80	46	67	12	65	59	52	47
15	75	72	51	24	70	61	61	52
$\bar{X}$	62.4	63.5	45.9	19.1	62.9	63.9	43.6	46.3
S_D	14.2	11.7	11.3	6.7	8.5	7.2	11.8	9.9
$S_{\bar{X}}$	3.7	3.0	2.9	1.7	2.2	1.9	3.0	2.6
	p > 0.05		p > 0.05		p > 0.05		p < 0.05	

A = Without antigen B = With amoebic antigen

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Without antigen

Patients (62.4 ± 3.7); controls (62.9 ± 2.2)
p>0.05

With antigen

Patients (63.5 ± 3.0); controls (63.9 ± 1.9)
p > 0.05

An important decrease of the number of leukocyte has occured after washing of the slides. Patients showed a marked immune response to the amoebic antigen but the controls didn't.

Without antigen - after washing

Patients (45.9 ± 2.9); controls (43.6 ± 3.0)
p>0.05

With antigen - after washing

Patients (19.1 ± 1.7), controls (46.3 ± 2.6)
p<0.05

The decrease of adherent cells with amoebic antigen after washing was found statistically significant in patients. In this preliminary report, we suggest that there is a cellular immune response to amoebic antigen. LAI assay can be useful for the detection of this immune response. Further studies will be needed to establish whether there is a correlation between the clinical course and/or prognosis of that disease and cellular immune response to specific antigen.

DISCUSSION

The ability of leukocytes from sensitized donors to adhere to glass surfaces is reduced when cells are preincubated with antigen (3, 4, 6, 8). The use of defined antigens permit an evaluation of this phenomenon with respect to its relevance to the immune response (5, 8). The assay method was described originally by Halliday and Miller (3). The LAI depends primarily on the presence of T lymphocyte and is uniquely suited to the assay of cell-mediated immunity in human (6, 8). The method is of peculiar interest because it appears to be specific to the detection of tumor immunity (2, 3, 4, 5).

This is the first report about the LAI in amoebiasis. We have studied the immune response to amoebic antigen in patients with amoebiasis. Persons infected by E. histolytica may harbor the parasite without any intestinal or extra-intestinal symptoms (10).

The role of immune system in the inflammatory response to E. histolytica has not been studied but early antibody production and retarded delayed hypersensitivity are in accordance with the observed cellular infiltrates.

The antigens used at present are mainly whole cells and homogenates in addition to soluble and particulate fractions (7, 10). Similarly we have prepared a soluble antigen from the whole cells and homogenates.

Delayed hypersensitivity to E. histolytica has been measured by delayed skin reaction in vivo and MIF production of peripheral cells in vitro (10). We could not apply skin test since we had no standard amoebic antigen. LAI test in similar MIF but the former is more simple and practical than the other. Large variations have been found in blast transformation and MIF production. The discrepancies may be technical in nature since different antigen preparations have been used. Many authors found that results of skin tests and test for MIF production were negative shortly after the onset of symptoms and before the initiation of therapy but become positive after patients were discharged from the hospital (10). We couldn't observed this initial unresponsiveness, but, we have found a significant immune response to amoebic antigen.

To our opinion LAI test is a rapid and sensitive procedure for the demonstration of cell mediated immunity in amoebiasis in vitro.

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