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Study of lymphocyte transformation response (LyTR) by using PHA, Con A and Pokeweed mitogens in children infected with Norovirus

دراسة الاستجابة للتحول اللمفي باستخدام المشطرات (الفيتوهيماكلوتينين) و (الكونكانافالين) و (البوكويد) عند الأطفال المرضى المصابين بالنوروفيرس .

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Abstract :

There are several agents known to be capable of inducing blast transformation of small lymphocytes. The mitogens phytohaemagglutinin (PHA), concanavalin A (con A) and pokeweed mitogen (PWM) are good examples of such agents . In this study we used The heparinized blood from each of the 45 sample was examined for immuno-suppressive activity.

peripheral blood lymphocytes stimulated with phytohemagglutin mitogen (100 ,150,250 µg/ml) , Concanavalin A mitogen (150 µg/ml) and Pokeweed mitogen PWM (150,300 µg/ml) were severely reduced in acute infantile diarrhea with Norovirus Infection in comparing with acute infantile diarrhea without Norovirus Infection and control group . acute phase heparinized blood appears to block initial steps in lymphocyte transformation.

الخلاصة :

توجد عدة عوامل معروفة قادرة على حث التحول اللمفي لخلايا اللمفية الصغيرة وأفضل مثال لهذه العوامل التي تعمل كمشطرات لمفية (الفيتوهيماكلوتينين) و(الكونكانافالين) و (البوكويد) وفي هذه الدراسة استخدمنا عينات الدم تم معاملتها بالهيبارين من 45 عينة وتم فحص الفعالية التثبيطية المناعية.

تم تحفيز الخلايا اللمفية في عينات الدم باستخدام المشطرات (الفيتوهيماكلوتينين) بتراكيز(100 و 150 و 250 مايكروكرام\مل) والمشطر (الكونكانافالين) بتركيز(150 مايكروكرام\مل) والمشطر (البوكويد) بتراكيز(150 و 250 مايكروكرام\مل) اختزال حاد في الفعالية التشطيرية في عينات دم الأطفال المرضى المصابين بالنوروفيرس والإسهال بالمقارنة مع عينات دم الأطفال المرضى المصابين بالإسهال من دون فيروس (النوروفيرس) ومجموعة السيطرة. عينات الدم طور الإصابة الحادة التي تم معاملتها بالهيبارين أظهرت حجز خطوات البدء في عملية التحول اللمفي.

Introduction:

Acute infection with norovirus results in reversible histopathologic lesions in the jejunum but not the stomach or rectum and manifests with vomiting and diarrhea. Changes appear within 24 hours of viral challenge, remain through the height of the illness, and persist for a variable time after the illness[(1)]. Intestinal villi appear blunted, but the mucosa remains intact. On electron microscopy, epithelial cells are intact, but microvilli are shortened and have widened intercellular spaces[(2)].

The major mode of transmission is fecal-oral spread, usually through consumption of a fecally contaminated vehicle (either food or water) , because the infectious dose is as low as 100 viral particles, low-level contamination of food and water can lead to outbreaks. A food vehicle may be fecally contaminated at its source (such as oysters harvested from contaminated waters or fruits and vegetables grown in contaminated environments) . Transmission of noroviruses occurs year-round, but higher disease incidences occur in the winter months in temperate climates [(3)].

Pokeweed mitogen PWM-transformed peripheral blood lymphocytes were shown to have a spectrum of morphologic differentiation from numerous large lymphoblasts to relatively few plasmacytoid cells. In addition, they were found to have enhanced intracellular Ig synthesis (2.5-fold increase over baseline resting state) but no significant rise in secreted Ig, thus exhibiting enhanced Ig production but retaining a lymphocyte-like Ig kinetic profile. This suggests that the PWM-transformed peripheral blood lymphocyte represents only a partial differentiation toward the mature antibody-secreting plasma cell [(4)].

There are several agents known to be capable of inducing blast transformation of Small lymphocytes. The mitogens phytohaemagglutinin (PHA), concanavalin A (con A) and pokeweed mitogen (PWM) are good examples of such agents [5].

Recently it has been shown that a heat-stable lipopolysaccharide extract obtained from Escherichia coil will induce a transformation of B lymphocytes in the mouse(6)

The aim of this study is to inducing blast transformation of lymphocytes in acute infantile diarrhea by three types of mitogens .

Materials and Methods :

The peripheral blood lymphocytes from heparinized blood from each of the 45 sample was collected from (Babylon Hospital for Women and Children) and examined for immuno-suppressive activity.

In vitro cell-mediated immunity tests :

stimulation of peripheral blood lymphocyte (PBL) from normal children were isolated on ficoll and incubated under optimal culture conditions. In brief, each culture contained PBL in 0.15 ml of RPMI-1640 medium supplemented with 10% heat-inactivated fetal calf serum, 15% pooled normal Heparinized blood or test Heparinized blood, 10 mM HEPES buffer (N-2-hydroxyethyl piperazine-NM-2-

ethane sulfonic acid), 20 mM L-glutamine, 100 U of penicillin, and 100 jig of strepto- mycin per ml (Centre of the cancer searchs and the medical genetic) . The following concentrations of mitogens in 0.05 ml of RPMI medium were added to each culture; (100, 150, 250 µg/ml) of PHA, (150 µg/ml) of ConA and (150, 250 µg/ml) of PWM (Centre of the cancer searchs and the medical genetic) .

Statistical analysis :

Statistical analysis were conducted to describe different variables and parameters in the research, and to describe relationship with each other as well. Calculation of mean value and standard deviation (SD) were made for immunological parameters.

The statistical significance of difference in mean of variable between more than two groups was assessed by ANOVA test .Probability values of $P<0.05$ were considered statistically significant.

Results:

Table (1): lymphocyte transformation by using PHA100 µg/ml mitogen among acute infantile diarrhea with Norovirus .

Groups	No.	Mean	Std. Deviation	Std. Error
Acute infantile diarrhea with Norovirus Infection	16	38.0233	1.8247	1.0535
Acute infantile diarrhea without Norovirus Infection	19	44.6333	1.4572	.8413
Control	10	55.4067	4.6836	2.7041
Total	45	46.0211	8.0368	2.6789

Table (2): lymphocyte transformation by using PHA150 µg/ml mitogen among acute infantile diarrhea with Norovirus .

Groups	No.	Mean	Std. Deviation	Std. Error
Acute infantile diarrhea with Norovirus Infection	16	41.7400	1.7536	1.2400
Acute infantile diarrhea without Norovirus Infection	19	45.7500	.3536	.2500
Control	10	56.4850	3.5143	2.4850
Total	45	47.9917	7.0433	2.8754

Table (3): lymphocyte transformation by using PHA 250 µg/ml mitogen among acute infantile diarrhea with Norovirus .

Groups	No.	Mean	Std. Deviation	Std. Error
Acute infantile diarrhea with Norovirus Infection	16	47.5300	.5233	.3700
Acute infantile diarrhea without Norovirus Infection	19	52.0150	1.1243	.7950
Control	10	60.2500	1.3435	.9500
Total	45	53.2650	5.8280	2.3793

Table (4): lymphocyte transformation by using Con A150 µg/ml mitogen among acute infantile diarrhea with Norovirus .

Groups	No.	Mean	Std. Deviation	Std. Error
Acute infantile diarrhea with Norovirus Infection	16	44.5300	1.0889	.7700
Acute infantile diarrhea without Norovirus infection	19	48.4800	.5940	.4200
Control	10	54.6750	1.3789	.9750
Total	45	49.2283	4.6485	1.8977

Table (5): lymphocyte transformation by using Pokeweed150 µg/ml mitogen among acute infantile diarrhea with Norovirus .

Groups	No.	Mean	Std. Deviation	Std. Error
Acute infantile diarrhea with Norovirus Infection	16	41.7100	2.7436	1.9400
Acute infantile diarrhea without Norovirus infection	19	46.6400	3.5072	2.4800
Control	10	54.3600	4.1861	2.9600
Total	45	47.5700	6.3241	2.5818

Table (6): lymphocyte transformation by using Pokeweed 300 µg/ml mitogen among acute infantile diarrhea with Norovirus .

Groups	No.	Mean	Std. Deviation	Std. Error
Acute infantile diarrhea with Norovirus Infection	16	43.9150	3.6699	2.5950
Acute infantile diarrhea without Norovirus infection	19	51.8950	1.5486	1.0950
Control	10	59.2850	2.2981	1.6250
Total	45	51.6983	7.1764	2.9297

Discussion :

Our study included peripheral blood lymphocytes stimulated with phytohemagg-lutin mitogen (100,150,250µg/ml) was reduced in acute infantile diarrhea with Noro- virus Infection in comparing with acute infantile diarrhea without Norovirus Infection and Control group [table 1,2,3] .

A decreased proliferative response to Pokeweed mitogen has been shown lymphocyte hyporesponsiveness to Pokeweed mitogen also appears to correlate with an impaired proliferative response to cytomegalovirus (CMV), Candida, and tetanus toxoid [7] .

peripheral blood lymphocytes to stimulation with phytohemagglutinin, and pokeweed mitogen was severely reduced, as was the ability of peripheral blood lymphocytes to respond to allogenic and xenogenic histocompatible antigens [8] .

in other study [9] founds changes in the responsiveness of Aotus monkey lymphocytes to mitogen stimulation with PHA and ConA during the course of *P. falciparum* infection (Aotus monkeys with low-grade parasitemias (<10%) showed no significant alterations in cell-mediated immune responses as judged by mitogen stimulation; however, a significantly depressed response of peripheral blood lymphocytes (PBL) to PHA and ConA stimulation was observed in animals with parasitemias between 25 and 50% .

peripheral blood lymphocytes stimulated with ConcanavalinA mitogen(150) and Pokeweed mitogen PWM (150,300) were reduced in acute infantile diarrhea with Norovirus Infection in comparing with acute infantile diarrhea without Norovirus Infection and Control group [table 4,5,6] .

In agreement with [10] we too found that LyTR to Pokeweed mitogen was significantly lower in the patients with AIDS.

Since serum inhibitors of T-cell responsiveness have been reported in several infectious diseases .it seemed possible that heparinized blood from acutely infected animals might have a suppressive effect on lymphocyte transformation [(11)] .

The lymphocyte transformation response (LyTR) to Pokeweed mitogen is significantly depressed in many patients with early HIV infection while the response to phytohaemagglutinin (PHA) remains preserved until late in the disease [(12)] .

in other study [(13)] on blast transformation of lymphocytes from diabetics and nondiabetics was evaluated after adding insulin at various concentrations. Responses to phytohemagglutinin-P (PHA-P), concanavalin-A (CON-A) and pokeweed mitogen (PWM) were measured in the presence of exogenous insulin added in physiologic increments of 0, 10, 20, 30, and 40 microunits of activity per ml of culture medium. Blast transformation in diabetics never reached the leve of blast transformation in nondiabetics. It is hypothesized that there is a cellular defect in either membrane receptors or intracellular metabolic pathways which accounts for the decrease in diabetic lymphocyte blast transformation.

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