Immunological response of diarrheal patients with determination of mice immunological state against some enteric bacteria

AbdalnabiJwaiedAbid
Dept. of Biology , Coll. Of science for women , Univ. of Babylon .

Abstract :

Blood and stool cultures of gastrointestinal patients reveals two major types of bacteria , *Citrobacterfreunds and Salmonella typhi* . *Citrobacterfreundi*form high rate of these isolates . Somatic and flagellar antigen of *S. typhi* , outer membrane protein (OMPs) and lipopolysaccharides (LPS) antigens of C. freundi were isolated .

Antibody and complement components titers show enhancement during early stage of infection . Mice that infected with bacteria reveals enhancement of immunological response represented by increasing of migration inhibition index value (MIF) and phagocytosis of infectious agents . Skin test also done for immunized mice with bacterial antigen Group of mice that Immunized with Somatic antigen of Salmonella and group that immunized with OMP of Citrobacter give a positive results for Delayed type hypersensitivity test .

Introduction :

Gastrointestial infection caused by different pathogenic agents such as viruses bacteria and parasites that may be transmitted in food , drinking water or by fecal- oral route . Human diarrheal symptom form a big problem worldwide especially with children ,children under five years are more susceptible to these infections(1). World health organization (WHO) estimated that out of the nearly two billion annual diarrhea diseases three million end fatally (2) .

Diarrhea is the chief symptom associated with enteric bacilli .Enterobacteiacae , one of the most causative agents ,consist of various bacterial genera some of their represents normal flora , moreover , many members of this family forms effective pathogenic bacteria to human and animals (3). Citrobacter is one of these effective enterbacteriacae genera that cause alone or with other bacteria miserable's for human (4) .

Identification of bacteria depends on several characteristics and techniques, *Citrobacterfreundii* characterized by a critical features for identification such as their ability to produce urease and the presence of some virulence factors that enhanced their efficacy in adherence and pathogenicity .presence of fiambriae as an adhesive bacterial component forms an important factors that elicit adherence of bacteria with mucosal epithelial cells of host enteric and urogenital tract (3).

Citrobacterfreundii characterized by their antigenic diversity , outer membrane protein (Omp) is one of the most important bacterial antigen participate in stimulating of host immune response (5)

Lipopolysaccharides (LPS) , the critical component of gram negative bacteria ,provided bacteria with endotoxin that form acritical factor of bacterial pathogenicity and its ability to induced immune response (6) .

Laboratory animals were used as a model for bacterial infection , many researchers utilized different gram negative bacteria for animal infection , rabbit , rat ,gueinia pigs , hamsters and other experimental animals were used for various kinds of study, Immunological investigations on experimental animals deal with determination of markers that contributed in host defense against these pathogens . (7; 8).

Material and Methods :

Samples : Stool and blood samples of diarrheal and gastrointestinal patients were collected from patients admitted to Hilla health centers during October 2010-june 2011 according to symptoms of these diseases . Bacteriological study includes culturing of stool and blood specimens with selective and differential media . Biochemical investigations were done for bacterial identification (9; 10) .

Bacterial antigens preparation :

Somatic and flagellar antigens(O and H Ag) were isolated from Salmonella using 0.3 -0.6 formal saline (11).

Outer membrane protein antigen (OMPs) of *Citrobacterfrundi*i were prepared using cold Hepes buffer solution (12).

Lipopolysaccharides antigen (LPS) of *Citrobacterfrundii*were prepared according to Learn et al technique (13) .

Animals : White albino mice 17-25 gm weight were reared in standard cages using suitable feeds and clean water . one group of mice were infected with Salmonella typhi another group were infected with *Citrobacterfrundii* , infected of mice were done by given the bacteria orally for 1 week and the mice were follow up searching for bacteria with mice feces .. Other group of mice were divided into 5 subgroup G1 immunized with somatic antigen , G2 immunized with flagellar antigen , G3 immunized with OMPs and G4 immunized with LPS antigen intradermally , booster doses of the same antigens were done after1 ,2and 3 weeks ,immunized mice were dissecting at the 4 week of immunization(14) . Blood were collected from heart directly then used for immunological investigations .

Immunological assay :

Blood and sera of patients were used for hematological and immunological investigations including W.B.C. differential count , phagocytic activity , immunoglobulins , complement titers , leukocytes inhibitor factor (LIF).

Blood and sera of immunized animals were investigated immunologically with NBT test, macrophage inhibitor factor and skin test (14) .

Statistical analysis

All hematological and immunological data of human and animals were analyzed statistically using complete randomized design (CRD) followed by LSD (15) .

Results and Discussion :

Bacteriological study of blood and stool specimens of diarrheal and gastrointestinal patients reveals many bacterial isolates , this study concerned with two types of bacteria *Citrobacterfrundii and Salmonella typhi* . Numbers of bacterial isolates varies with type of specimens and virulence efficacy , *Citrobacterfrundii* forms highest rate of these isolates while *Salmonella typhi* form less rate for both specimens (table 1) . Members of Enterobacteriacae including *Citrobacter and Salmonella* genera characterized by their highly ability to cause enteric infection in human and the symptoms of infection appears with certain days as a results of their enterotoxins activity (16) , furthermore , the surface components of bacterial cells elicited their invasive ability to the epithelium of enteric canal (17).

Table 1. Bacterial isolates from diarrheal infected children

Samples	Bacterial types	No. and % of isolates	
Blood	*Citrobacterfreundii*	5	8.3%
Stool	*Citrobacterfreundii*	8	20%
Blood	*Salmonella typhi*	4	6.6%
Stool	*Salmonella typhi*	2	5%

*Total no. of blood culture is 60 specimens and stool culture is 40 specimens

Many antigens of these bacteria were isolated including somatic and flagellar antigens for *Salmonella typhi* and outer membrane proteins (OMPs) and lipopoly saccharides (LPS) antigens of *Citrobacterfrundii* .Bacterial entero pathogens cause high rate of diarrheal cases (18) Bacterial antigens had effective role in inducing host immune responses through their ability in adherence and invasive to host mucous membranes , although some of these antigens enhanced cytotoxic activity and pathogenicity of these bacteria (19; 29).

The present study reveals also an increased in immunoglobulins and complement titers . IgM concentration shows a highest rate with the diarrheal patients while serum C3& C4 concentrations appears high with gastroenteritis patients (table 2) .Serum antibodies forms an important tools in protection and host defenses against invasive pathogens ,since *Citrobacter* and Salmonella were enteric bacteria so IgM and IgA were the most inducing immunoglobulins through their colonization, these pathogens release toxins which binds to the intestine and cause diarrhea , others damage the intestines them self by their direct presence (21) . Complement components participated as an effective host defense mechanisms against pathogens , they acts as opsonins alone or with antibodies to elicit kill processes through inducing of inflammatory response resulting in diarrhea and protective antibody response (6) .

Table 2. Immunoglobulins and complement concentrations of patients sera.

Subjects	IgM	C3	C4
Diarrheal pat.	139.8 ±304	274 ±13.49	73.9±6.83
Gastrenteritis pat.	133.6± 5.05	299.6± 10.98	84.4± 5.87
Healthy (control)	110.6±4.81	122.8± 7.06	32.4±7.72

Phagocytic activity increased with the type of infections , reduction of nitrobluetetrazolium by poly morphonuclear cells increased with enteric infection and reach the higher rate (14 %)with diarrheal patients (table 3) . There are several components of bacterium correlated with inducing of humeral immune response that mediated with T-cell . Bacterial antigens presented to T-cell as a peptides through MHC II that binds antigens with T cell receptor of T helper1 , this process followed by activation of CD8+ (22).

Table 3. Nitrobluetetrazolium positive polymorphonuclear cells of gastroenteritis patients.

Subjects	NBT +ve PMNs % (M±S.D)
Diarrheal pat.	14 .6 ±1.5*
Other gastroenteritis dis.	10.7 ± 1.49
Healthy (control)	7.7± 1.63

,*= there is a significant differences at P> 0,05

Lymphocytes migration assay were done to evaluated the activity of T- cells , leucocytes inhibition factor value reveals a noticeable decline in gastrointestinal patients compared with their value in healthy persons .

Table 4. LIF value of gastroenteritis patients.

Subjects	LIF value (M± S.D)			
	OAg	Hag	LPS	OMPs
Diarrheal pat.	0.51±0.08	0.75±0.08	0.64± 0.10	0.50± 0.05
Other gastroenteritis pat.	0.56± 0.09	0.74±0.07	0.60 ±0.03	0.48± 0.03
Healthy (control)		0.89± 0.06	0.80± 0.05	0.78± 0.04

*each group consist of 5 animals. ,healthy control for O Ag =0.07±0.05

Phagocytic processes of experimental animals differs with type of antigens . Animals immunized with Outer membrane protein antigen (OMPs) show highest rate of phagocytosis , polymorpho nuclear cells positive for NBT test reach 16 and 11 % for group of mice treated with OMP and group of mice treated with H antigens respectively (Table 5) . There are two mechanisms involved both of which are diarrheagenic , first , bacteria can produce diarrhea by invading the bowel wall as in salmonella and shigella , second , the bacteria may float comfortable in the bowel lumen and secret toxins(that induce phagocytosis and killing processes (23) .

Table 5. NBT positive PMNs of immunized mice with bacterial antigens.

Groups	NBT +ve PMNs% (M± S.D)
G1(immunized mice with OAg)	12 .2±1.48
G2(immunized mice with Hag)	10.6± 0.89
G3(immunized mice with LPS)	15.6 ± 0.89*
G4(Immunized mice with OMP)	16 ± 1.22*
Control	8.8± 0.83

,*= there is a significant differences at P> 0,05

The present study deal also with detection of inhibition factor of macrophage migration .MIF value of immunized mice differ with type of antigens ,the highest value appears with group of mice that immunized with OMPs and O antigen 6.0 and 6.6 respectively (table 6) .

Table 6. MIF value of of immunized mice with bacterial antigens.

Groups	MIF value (M± S.D)
G1(immunized mice with OAg)	7.2 ± 0.83*
G2(immunized mice with Hag)	4.8± 0.83
G3(immunized mice with LPS)	6.2 ± 0.81*
G4(Immunized mice with OMP)	6.2 ± 0.80
Control	4.0 ± 1

,*= there is a significant differences at P>

Injection of animals with antigens induce hypersensitivity that differs with type of antigens , *Salmonella* O antigen and*Citrobacter* OMPs antigen reveals a noticeable efficacy to induce delayed type hypersensitivity . The thickness diameter reach 13 and 12 mm for O Ag immunized mice and OMPs immunized mice respectively (table 7) .

Table 7. Delayed type hypersensitivity value of immunized mice with bacterial antigens.

Groups	DTH(thickness diameter mm)
G1(immunized mice with OAg)	6.0± 0.70*
G2(immunized mice with Hag)	5.6 ± 0.89
G3(immunized mice with LPS)	6.2 ± 0.44*
G4(Immunized mice with OMP)	6.6 ± 0.89*
Control	3.6 ± 1.14

Each group consist of 5 animals ,*= there is a significant differences at P> 0.05

- Cellular immune response represented in this study by migration inhibition index and hypersensitivity was inducing as a results of bacterial infection and immunization of mice with bacterial antigens particularly with immunosuppressive patients (24).

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