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ASSESSMENT OF CELL MEDIATED IMMUNITY AGAINST *SALMONELLA TYPHI* ANTIGEN IN GUINEA PIGS FOLLOWING INTRAPERITONEAL INJECTION OF SENSITIZED SPLEEN CELLS

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ABSTRACT

In an assessment of cell mediated immunity against Salmonella typhi antigen in Guinea pigs following their intraperitoneal injection with sensitized spleen cells. The results were revealed a high level of cell mediated immunity against Salmonella typhi antigen in the Guinea pigs intraperitonealy received the sensitized spleen cells ($5x10^8$/ml). The level of cell mediated immunity was detected using delayed type hypersensitivity test, Macrophages migration inhibition test and Erythrocytes rosette test.
The normal (nonsensitized) spleen cell recipient group (control group) was showed no level of cell mediated immunity using these tests.

INTRODUCTION

Adaptive transfer of cell mediated immunity to specific antigen in human was first demonstrated by Lawrence (1), who showed that transfer of whole viable lymphocytes from a normal tuberculin skin positive donor to a normal skin test negative recipient resulted in a conversion of the recipient to skin test positive.
Lawrence (2) demonstrated that delayed cutaneous hypersensitivity responsiveness to Streptococcal M substances could also be transferred by Soluble extracts of leukocytes and termed the factor responsible for this phenomenon transfer factor, the important immunological effects are the antigen specific conversion of delayed type hypersesitivity and production of lymphokines (3,4).
The successful transfer of delayed skin sensitivity, also was done in mice using spleen cells sensitized against other antigenic materials, such as a particulate antigen (Candida) and soluble protein antigens (ferritin, cytochrome C, horse radish peroxidase and purified protein derivatives) (5).
Cell mediated immunity could be transferred from sensitized mice to nonsensitized, using immune spleen cells against Rhodococcus equi (6). Also, by using immune peritoneal macrophages against Salmonella typhimurium and Salmonella enteritidis (7).
Because Salmonella typhi is facultative intracellular pathogens, cellular immunity specifically immne T lymphocytes and macrophages were proposed as the most important defense mechanism, for this reason, this study was aimed to evaluate the role of sensitized spleen cells of the Guinea pigs in transferring cell mediated immunity against whole killed Salmonella typhi antigen (local typhoid vaccine), using delayed type hypersensitivity-skin test, Erythrocyte-rosette test (E-rosette test) and macrophages migration inhibition test.

MATERIALS AND METHODS

Guinea pigs:

Eighteen adult Guinea pigs, weighing 400-500gm each, supplied by AL-Kindi Company of Veterinary drugs and Vaccine production were reared together on

concentrated food. Blood samples were taken from all the animals in order to test level of antibiodies against Salmonella typhi.

The animals were divided into 4 groups.

First group; immunization group (5 animals):

The immunization procedures were done by subcutaneous injection of the animals with 0.25ml of killed whole cell vaccine-phenol preserved containing 10^9 bacterial cell/ml.

Booster doses of 0.25 and 0.5ml of vaccine were given subcutaneously on the 3rd and 5th week postinoculation respectively.

Second group; control group (5 animals):

Similarly, was injected with sterile phosphate buffer saline.

At the 14th and 21st days after the second booster dose a delayed type hypersensitivity-skin test and macrophages migration inhibition test were done on immunization group and control group according to Dham and Thompson and Weir methods (8,9), to check the level of cell mediated immunity and immunity transfer by for preparation of sensitized and non-sensitized spleen cells.

Sensitized spleen cells were taken from the immunized group of animals which showed high level of cell mediated immunity against Salmonella typhi sonicate soluble antigen (DTH-skin test was > 5mm and migration index (MI) < 0.80).

Similarly, normal (non-sensitized) spleen cells were taken from the control group (2nd group).

The spleen cells from each animals in both groups were surgically taken-out in RPMI-1640, containing 10% fetal calf serum, and after trimming off all the adherent tissue, cut into small pieces, minced and teased on a sterile stainless sieve to obtain single cell suspension.

The leukocytes from the spleen cell suspension were made free from erythrocytes by treatment with 0.83% ammonium chloride for 10 minutes, after that the spleen cells suspension was washed 3 times by phosphate buffer saline and checked for viability using 0.2% trypan blue dye.

The number of spleen cells per ml of phosphate buffer saline was adjusted to $5X10^8$ cell/ml and used as viable cells for immunity transfer by intraperitoneal injection of the third group of animals (sensitized cell recipient group, containing 4 animals). Similarly, normal spleen cell suspension was prepared and intraperitoneally injected into the fourth group of animals (normal spleen cell recipient group, containing 4 animals).

After the injection of sensitized and normal spleen cells, an assessment of the cell mediated immunity was done in these two groups, using DTH-skin test which was done after 24hrs and MIF Test was done during 5th to 14th days postinoculation of spleen cells. Also, Erythrocytes-rosette test was done during this period according to Braganza etal. Technique (10)

RESULTS

This study was revealed the following findings:

Sensitized spleen cell recipient group; This group of animals was showed high level of cell mediated immunity in compare to the normal cell recipient group (control group), *using the following tests:*

1. DTH-skin test:

Marked skin reactive areas were seen in sensitized spleen cell recipient group, the mean diameters of reactive areas were (>5mm), 8.75±1.708mm, 6.5±1.291mm and 5.0±816mm after 24hrs of inoculation of concentrated Sonicate antigen (400, 40, 4 μg/ml) respectively, and then gradually decreased thereafter (table –1). The normal spleen cell recipient group (negative control) showed skin reactive areas, with mean diameters < 5mm (3.25±1.258, 2.25±1.258 and 1.75±0.957) after 24hrs of using concentrated Sonicate antigens (400, 40, 4 μg/ml) respectively.

2. Macrophage migration inhibition test:

Macrophage migration inhibition test (MIF) activity was determined in all animals, intraperitoneally received the sensitized spleen cells. The mean indices of macrophages migration were <0.80 (0.252±0.260, 0.427±0.212 and 0.669±0.348) at the different concentrations of Sonicate antigen (400, 40, 4 μg/ml) respectively (Table-2), whereas, the mean indices of macrophages migration were >0.80 (0.847±0.285, 1.530±0.736 and 2.249±1.732) in the normal spleen cell recipient group against different concentrations of Sonicate antigen (400, 40, 4 μg/ml) respectively.

3. Erythrocytes-rosette Test:

E-rosette formation active of and total T-lymphocytes were increased in all animals received sensitized spleen cells against Salmonella typhi antigen. The mean numbers of E-rosette formation active and total T-lymphocytes were 0.625±0.250 and 0.750±0.288 respectively before treatment.It increased to a mean of 12.250±2.872 and 13.500±3.109 for active and total T-lymphocytes respectively in sensitized spleen cell recipient whereas, the mean numbers of E-rosette formation active and total T-lymphocytes were 0.625±0.250 and 0.750±0.288 respectively before treatment. It increased to 1.250±0.500 and 1.500±0.577 respectively in the normal spleen cell recipient group (Table-3).

Table-1: DTH-skin test in different recipient groups of G. pigs following I/D injection of S. typhi Ag.

Group	Diameters mm	Time/hours											
		24 hrs.				48 hrs.				72 hrs			
		Ag concent µg/ml			PBS	Ag concent µg/ml			PBS	Ag concent µg/ml			PBS
		400	40	4		400	40	4		400	40	4	
Sensitized spleen cell recip.	Range	7-11	5-8	4-6	0	6-9	4-7	3-5	0	4-7	3-6	3-4	0
	Mean	8.75	6.5	5.0	0	7.0	5.5	4.0	0	5.5	4.5	3.25	0
	±	±	±	±		±	±	±		±	±	±	
	S.D	1.708	1.291	0.816	0	1.414	1.291	0.816	0	1.291	1.291	0.5	0
Normal spleen cell recip.	Range	2-5	1-4	1-3	0	1-3	0-3	0-2	0	0-2	0-2	0-1	0
	Mean	3.25	2.25	1.75	0	2.0	1.25	0.75	0	1.0	0.5	0.25	0
	±	±	±	±		±	±	±		±	±	±	
	S.D	1.258	1.258	0.957	0	0816	1.258	0.957	0	0.816	1.00	0.50	0

PBS : Phosphate Buffer Saline

ation inhibition indices (MI) following various treatments in G. pigs in response to S. typhi Ag.

Animal No.	Ag concent µg/ml			PHA (10 µg/ml)
	400	40	4	
1	0.640	0.640	1.000	0
2	0.140	0.562	0.765	0
3	0.081	0326	0.734	0
4	0.148	0.179	0.179	0.213
D	0.252±0.260	0.427±0.212	0.669±0.348	0.053±0.106
1	0.694	1.000	1.361	0
2	1.265	1.562	1.562	0
3	0.790	1.000	1.234	0.197
4	0640	2.560	4.840	0
D	0.874±0.285	1.530±0.736	2.249±1.732	0.049±0.098

ohemagglutinine

Table-3: Mean percentage values of active and total T-lymphocytes in the recipient groups (E-rosette Test).

Pretreatment period		Post treatment			
		Sensitized spleen cell recip.		Normal spleen cell recip.	
AT[1]	TT[2]	AT	TT	AT	TT
0.625±0.250	0.750±0.288	12.250±2.872	13.500±3.109	1.250±0.500	1.500±0.577

[1] AT= Active T lymphocytes. %

[2] TT= Total T lymphocytes. %

DISCUSSION

The major host defense mechanism for Salmonella infection is controversial, but it is clear that both humoral and cell mediated immunity responses are important (11). Because Salmonella are facultative intracellular pathogens, cellular immunity specifically immune macrophages and T lymphocytes is proposed as the most important defense mechanism (12).

A similar findings were correlated with the present study in which sensitized spleen cells had ability to transfer cell mediated immunity against the Salmonella typhi antigen, detected by DTH-skin test and MIF test. Histologically, spleen tissue was composed of T cells (85%), responsible for lymphokines production, B cells (15%) responsible for antibody production. Other cell type was also present in spleen tissue such as dendritic cells (macrophages) responsible for the antigen processing and some lymphokines production (13) and therefor following the injection of the animals with antigen (Salmonella typhi antigen) in the present study, the spleen cell became sensitized and had the ability to transfer the cell mediated immunity to the recipient group which gave DTH-skin reactive areas >5mm diameter and migration indices (MI)<0.80. similarly, the sensitized peritoneal macrophages and spleen cells gave protection against Salmonella typhimurium and Salmonella enteritidis (7) and against other type of microorganisms such as Rhodococcus equi (6). Also, similar findings were detected following sensitization of spleen cells with particulate antigen (Candida) and Soluble protein antigens (Ferritin, cytochrom C, horse radish peroxidase and purified protein derivatives) (5) and also with tuberculin (14) using sensitized lymphocytes of tuberculin hypersensitive Guinea pigs.

تقييم للمناعة الخلوية ضد مستضد عصيات التايفوئيد في خنازير غينيا بعد حقنها بخلايا الطحال المحسسة داخل تجويف البطن.

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الخلاصة

في تجربة صممت لتقييم مستوى المناعة الخلوية ضد مستضد عصيات التايفوئيد في خنازير غينيا بعد حقنها بخلايا طحال محسسة داخل تجويف البطن و بجرعة ٥ ١٠x ^ خلية/مللتر، حيث دلت النتائج إلى وجود مستوى عالي للمناعة الخلوية ضد مستضد عصيات التايفوئيد في خنازير غينيا بعد حقنها بخلايا الطحال المحسسة. وقد قيست المناعة الخلوية باستعمال الاختبارات التالية:

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