



ROLE OF BCG VACCINE IN BACTERIAL DISSEMINATION, PATHOLOGY AND IMMUNIZATION AGAINST ENTEROPATHOGENIC *ESCHERICHIA COLI* AND THERE CHALLENGE INFECTION IN WHITE ALBINO MICE

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ABSTRACT

This study aimed to determine the role of BCG in immunization of white mice against Enteropathogenic *Escherichia coli* (EPEC) and their role against challenge infection and pathology with this microbial agent. EPEC was isolated from diarrhea of children, routinely diagnosed and serotyping was identified as 0119 EPEC. Then three groups of mice (30 each group) were taken, first group immunized with whole sonicated EPEC Ag, the second group were immunized with whole sonicated EPEC Ag and BCG Vaccine, the immunization were occurred at two doses 14 days intervals, third group were injected with phosphate buffer saline (pbs- control group). Results revealed at 28th days post immunization, increase in DTH- skin test thickness at 24, 48 hrs in group immunized with whole sonicated EPEC Ag and BCG (group2) comparable to group 1 (immunized with whole sonicated cell Ag alone and group 3 (control group). Also humeral immune (HI) response elevated in group 2 comparable to group 1 and control group 3. The HI response accompanied by elevation of IgG and IgM level in group 2 comparable to group 1 and control group 3. On another hand bacterial isolation were mild in group 2 and moderate in group 1 comparable to control group 3 following challenge dose infection with EPEC. Also more localized granulomatous lesion was seen in internal organs specially at 14th- 21th days post inoculation of challenge dose in group 2 comparable to group 1 and no lesion in control group 3.

KEYWORDS: Role of BCG in immunization & protection against EPEC 0119 challenge infection and pathology in mice

INTRODUCTION

Enteropathogenic *Escherichia coli* (EPEC) are the cause of sever and persistent infant diarrhea both in developed and in developing countries^[1]. It is a major medical problem with serial consequences in children less those have 3 months of age. In addition EPEC is important cause of morbidity and mortality in weaned rabbits^[2]. EPEC is highly pathogenic in neonatal calves^[3] and frequently isolated from swine with recurrent post weaning diarrhea^[4] and also had a diarrheagenic role in dogs^[5]. *Bacillus Calamite* Guerin (BCG) is alive attenuated vaccine derived from strain of *mycobacterium bovis*. BCG vaccine is a part of global expanded program for immunization^[6] is considered a safe vaccine used in treatment and control of some bacterial diseases such as T.B and through its role in immunization. Also it used in immunization against some tumors suchas bladder cancer. For all reasons mentioned above this study aimed at:

- 1-Study the role of BCG in immunization against EPEC together with whole sonicated EPEC Ag.
- 2-Study the bacterial dissemination of EPEC in immunized animals under effect of BCG following challenge infection with EPEC.
- 3-Study the pathological findings in immunized animals and under effect of BCG following challenge infection of mice with EPEC 0119.

MATERIALS & METHODS

Three groups of albino white mice were used in this study. First group: immunized at first day and 14th day with whole sonicated EPEC 0119 Ag (0.1ml) S/c prepared according to^[7]

2ndgroup: immunized at first days and at 14th day with whole sonicated EPEC 0119 Ag (0.1ml) S/c together with 0.1 m BCG 1/d

3rdgroup: injected with phosphate buffer saline (pbs) 0.1 ml S/c at first day and 14th day. In all animals groups of immunization at 28th day of immunization, Delayed type hypersensitivity (DTH) skin test were done^[8]. Blood samples were collected for estimation of humeral immunity^[8] and IgM and IgG levels were estimated by serum electrophoresis^[9] at 30th day of immunization. At 30th day post immunization, all animals groups were injected with 0.1ml of 10x9¹⁰CFU/ml (10LD50) of EPEC 0119, LD50 determined according to^[10], 5 animals from each group were sacrificed at 7th, 14th and 21th day, 28th days post challenge dose and bacterial isolation from internal organs were done and pieces of lesions in internal organs taken for histopathology^[11].

RESULTS & DISCUSSION

Delayed type Hypersensitivity (DTH) skin test:

The results showed increase thickness of foot bad of mice (1.98± 0.1, 2.14 ± 0.1) at 24 hrs and 48 hrs respectively in groups 2 (immunized with whole sonicated EPEC Ag and BCG Vaccine) comparable to group 1 (immunized whit whole sonicated Ag alone, 1.76± 0.4, 1.88± 0.1) at 24, 48 hrs respectively and no (Negative skin thickness in control group 3) (Table -3)

Indirect Hemagglutination test (IHA)

The results showed increase in antibodies titer (32 ± 0.1) in group (2) of mice immunized with whole sonicated

EPEC Ag and BCG comparable to group (1) immunized with EPEC Ag alone (16 ± 0.25) and in control group (3) 4 ± 0.2 . (Table -1)

Quantitative Antibodies type levels of electrophoresis

The results showed increase the level of IgG and IgM in group 2 immunized with whole sonicated EPEC Ag and

BCG vaccine, the levels were 12.92 ± 0.04 for IgG and 22.14 ± 0.08 for IgM comparable to the level of IgG and IgM 10.79 ± 0.12 and 14.39 ± 0.06 respectively in group 1 immunized with whole sonicated EPEC Ag alone whereas in control group (pbs) group 3 (9.55 ± 0.03 and 9.43 ± 0.02 respectively for IgG and IgM (Table – 1).

TABLE 1: DTH – Skin test, Antibodies titer and serum immunoelectrophoresis for IgG and IgM in groups of mice immunized with whole sonicated EPEC Ag & BCG and control

Immunological Tests	Whole sonicated EPEC Ag		Whole sonicated EPEC Ag & BCG Vaccine		Control (pbs)	
	24hrs	48hrs	24hrs	48hrs	24hrs	48hrs
DTH – skin test	Thickness of foot pad(mm)		Thickness of foot pad(mm)		Thickness of foot pad(mm)	
	1.76 \pm 0.4	1.88 \pm 0.1	1.98 \pm 0.1	2.14 \pm 0.1	0	0
Abs titer IHA Test	16 \pm 0.25		32 \pm 0.1		4 \pm 0.2	
Immunoelectrophoresis , level of IgG	10.79 \pm 0.12		12.92 \pm 0.05		9.55 \pm 0.03	
Immunoelectrophoresis , level of IgM	14.39 \pm 0.06		22.14 \pm 0.08		9.43 \pm 0.02	

DTH skin test was used when prior exposure to Ag had occurred, so at the re exposure of body to the same Ag resulted into swelling and indurations at the site of injection of the same Ag, the swelling and induration occurred as a result of mononuclear cells infiltrations (lymphocytes and macrophages) and edema at the site of Ag injection which more evident in this study at 24hrs & 48 hrs in group 2 (immunized with whole sonicated EPEC Ag and BCG vaccine) comparable to group 1 (immunized with whole sonicated EPEC Ag alone). The cellular reaction at the site of swelling and induration has been dependent on memory T cells and role of CD4⁺, CD8⁺ inducer for CMI^[12] both these cells proliferated at the site of swelling & induration in response to Ag re exposure, so high level of cytokines such as IL₁ from sensitized macrophages and IL₂ and interferon–gamma from sensitized helper cells (H1) both cytokines activate the macrophages and act as chemotactic factor for macrophages & lymphocytes^[13] to the site of induration & swelling a response to Ag inoculation, so the BCG vaccine gave important role in stimulation and potentiation of cellular immune response in Group 2 comparable to group 1 (immunized with whole sonicated EPEC Ag alone).

The results showed high level of antibodies detected by IHA test and the type of Abs were IgG and IgM detected by the electrophoresis in group 2 (immunized with whole sonicated EPEC Ag and BCG vaccine) comparable to low level in group 1 (immunized with whole sonicated EPEC Ag alone). These results belong to the stimulation and potentiation effect of BCG with whole sonicated Ag comparable to whole sonicated Ag alone in group 1, so the synergistic effect of both Ags enhance the high level of Abs (IgG, IgM) in group 2 comparable to group 1 through the induction of TH2 which aid in the synthesis high level of IgG and IgM through release IL4, IL5 both is

important for differentiation of B lymphocytes into plasma cells^[14] in addition, BCG act as potent stimulator for HI and CMI which more evident in this study in group 2 comparable to group 1, similar finding reported by^[15]. Also BCG act as inducer for IL4 from bone marrow precursor cells and B cells precursor, and enhance the development of Th2^[16] which support B cell maturation into plasma cells, and resulted into high level of Ab response. Also the high level of Ab (IgG and IgM) in the secondary immune response in group 2 comparable to group 1, related to that the BCG and whole sonicated EPEC Ag re exposure enhance high level Abs than in primary immune exposure, so the Abs were more rapidly released and highly elevated than in primary immune response, this attributed to Ag sensitized memory cells which proliferated and more H2 cells stimulated and increased maturation of B cells into plasma^[17] which more evident in this study in group 2 comparable to group 1.

Clinical and Bacteriological isolation

Both groups of mice (1, 2, 3) showed healthy animals during the course of experiment, the group 2 (immunized with whole sonicated EPEC Ag and BCG vaccine) give protection rate 100% comparable to group 1 (immunized with whole sonicated EPEC Ag alone and challenged with EPEC 0119 which give 90% protection rate comparable to non immunized group (infected with 10 LD50 EPEC 0119 strain in which all animal died during 48hrs post infection).

Bacteriological isolation: Mild bacterial isolates from internal organs of group 2 (immunized with whole sonicated EPEC Ag and BCG Vaccine) comparable to moderate bacterial isolates in group 1 (immunized with whole sonicated EPEC Ag alone) and control group 3, Pbs (Table-2).

TABLE 2: Bacterial isolation from internal organs of immunized with whole sonicated EPEC Ag, BCG vaccine and control group (pbs)

Groups	Spleen	Liver	Kidney	Lung	Hear	Brain
Group 1 whole sonicated EPEC Ag alone	++	++	++	++	+	+
Group 2 whole sonicated EPEC Ag BCG vaccine	+	+	+	+	-	-
Group 3 control (pbs)	-	-	-	-	-	-

Note: + mild bacterial isolates, ++ moderate bacterial isolates, - No bacterial isolates

The mild bacterial isolates indicated that some bacterial colonies spread from the injection site into the internal organs such as livers, spleen, lung, kidney & intestine, these organs were enriched with macrophages which activated under effect of immunization with whole sonicated EPEC Ag BCG vaccine, the activated macrophages under the effect of interferon gamma produced by natural killer cells^[18] in immunized group 2 can destroy the bacteria through phagocytosis process so resulted in to mild bacterial growth in these organs of group2 comparable to moderate bacterial growth in group 1 (immunized with whole sonicated EPEC Ag alone). Also memory cells in immunized groups directly differentiated into TH1 cells which limit the bacterial growth in activated macrophages, then releasing of tumor necrosis factor (TNF) and IL12 both affect on natural

killer cells to induce $INF - \gamma$ which increase phagocytic activity of macrophages and neutrophils^[19] through production of nitric oxide and super oxide radicals acting as a potent bacteriocidal activity. BCG act as immunostimulator and potentiator and enhancing phagocytosis and killing of EPEC by macrophages^[20] therefore, complete clearance of EPEC in group2 .

Pathological findings

No gross pathological lesion was seen in immunized group except hyperplasia of spleen in group1, 2 of immunization. Microscopically: Intestine showed extensive mucin secretion in their lumen, hyperplasia of goblet cells and mild mononuclear cells (lymphocytes, macrophages) and neutrophils in villarepith at 7th, 14th days post bacterial challenge also hyperplasia of peyer's patches (Fig-1) .

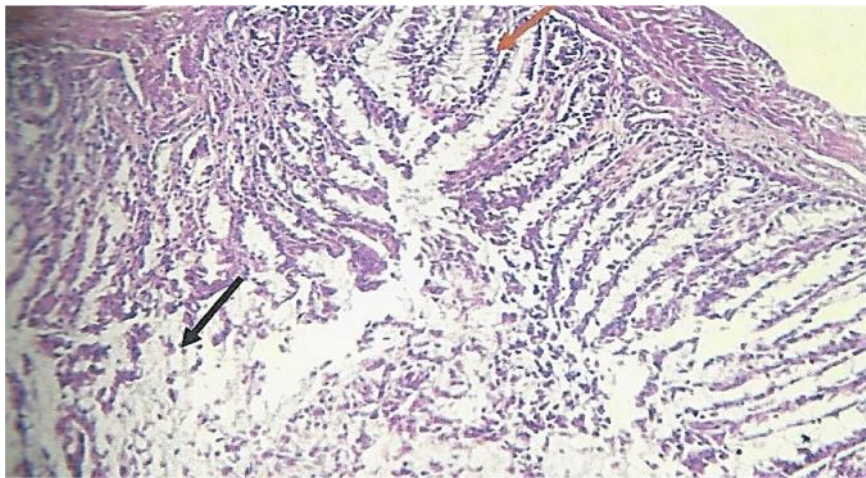


FIGURE 1: intestine showed hyperplasia of goblet cells and mucinous degeneration (H&E) x40

Liver: showed extensive infiltration of mononuclear and plasma cells in adjacent central vein and in portal areas (Fig-2). This cellular infiltration causing granulomatous reactions at 14th and 21th days post bacterial challenge.

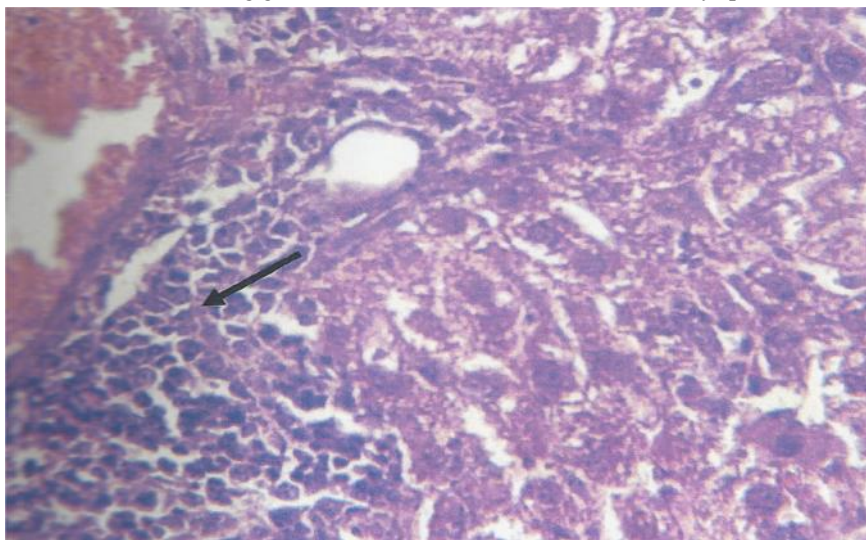


FIGURE 2: Liver showed early granuloma and congestion of central vein (H&E) x400

Lungs : showed extensive hyperplasia of peribronchial associated lymphoid tissue and congestion of blood vessels, emphysema and mononuclear cells infiltration in alveolar walls and adjacent to B.V. (Fig-3)

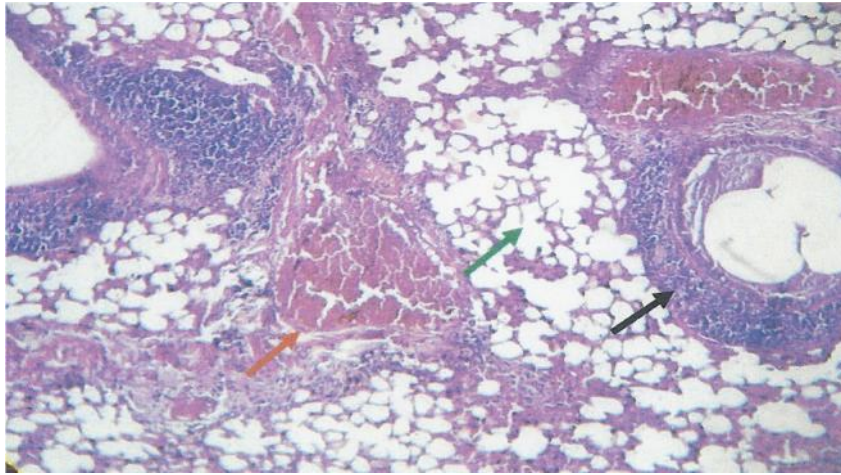


Figure 3): Lung showed hyperplasia of peribronchial lymphoid tissue and mononuclear cells infiltration in interstitial tissue (H&E) x40

Spleen: showed extensive hyperplasia of white pulp, infiltration of mononuclear cells and reticulated endothelial cell hyperplasia in red pulp & adjacent to B.V. Also extensive amyloid infiltration around the white pulp (Fig-4, 5)

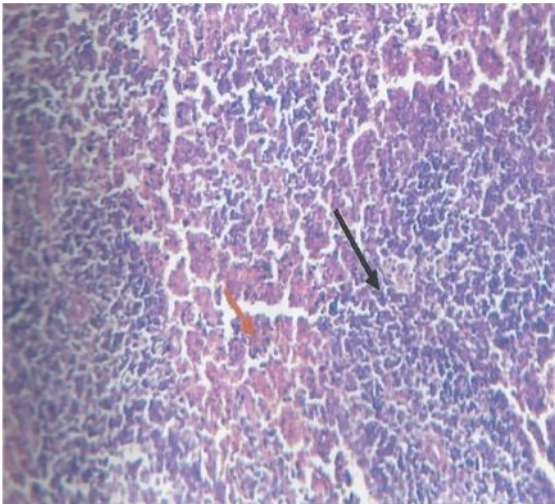


FIGURE 4: Spleen showed reactive hyperplasia of white pulp (H&E)x40

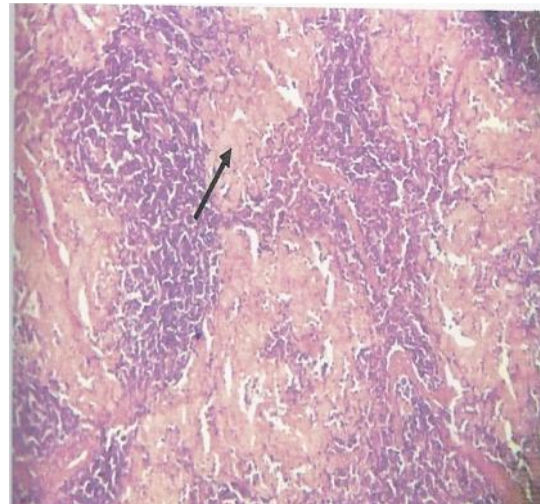


FIGURE 5: Spleen showed diffuse amyloidosis (H&E)x40

Kidneys: showed extensive cells infiltration of mononuclear cells and plasma in the interstitial renal tissue and adjacent B.V (Fig-6).

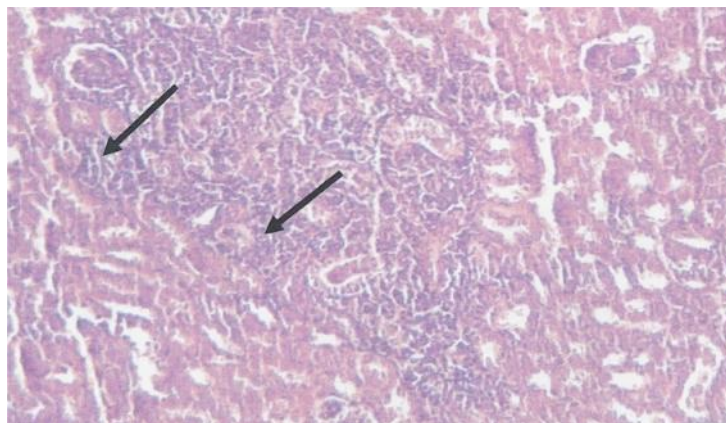


FIGURE 6: Kidney showed infiltration of mononuclear cells in interstitial renal tissue (H&E) x40

Heart : showed only mononuclear cells infiltration between muscle fibers (Fig-7)

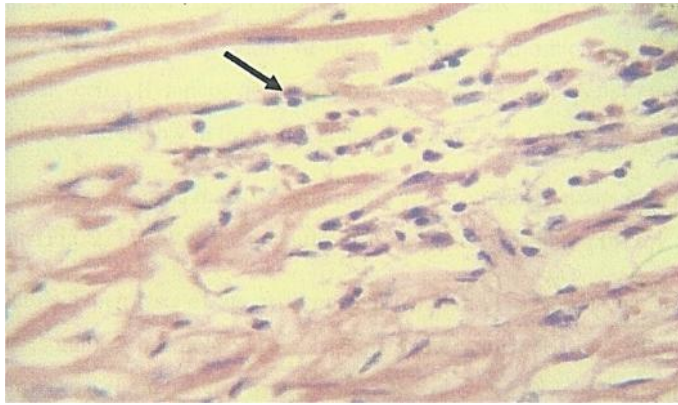


FIGURE 7: Heart showed infiltration of mononuclear cells between muscle fibers (H&E) x200
Control group (Pbs) showed normal histological sections.

In this study the pathological findings were confirmed with previous studies⁽²¹⁾ they referred that the phagocytic system is the earliest defence mechanism against microbial infection through their killing by neutrophils and macrophages which is more evident in this study in immunized group (1,2) in which all the internal organs showed extensive mononuclear cells and neutrophils infiltration causing a granuloma in some organs of immunized group with whole sonicated EPEC Ag and BCG vaccine, even Ag alone. BCG increase the number of phagocytes⁽²²⁾ which more evident in group 2 (immunized with whole sonicated EPEC Ag and BCG). Also BCG increase the capacity of bone marrow precursor cell for (lymphocytes and monocytes) releasing into body organs⁽²³⁾ which demonstrated in the immunization groups of mice. Also CD4⁺, CD8⁺ cells produce INF- γ in spleen and mucosal lymph nodes of mice which increase phagocytic activity of macrophages together with granuloma formation⁽²⁴⁾ which more observed in liver of immunized group in addition to hyperplasia of white pulp and mononuclear cells infiltration in the most internal organs adjacent to B.V⁽²⁵⁾, these inflammatory cells and granuloma indicate the CMI response which more evident in immunization groups, similar finding reported by⁽²⁶⁾. Amyloid were deposited around the white pulp, this filamentous protein commonly associated with continuous immune response against Ags in addition, the challenge bacterial dose (EPEC) act as a booster dose augment the activity of immune cells to produce inflammatory cytokines which stimulated hepatocytes to produce high level of serum associated amyloid over the ability of monocytes derived enzyme to degrade serum associated amyloid together with C-reactive protein a major acute phase protein produced in Kupffer cells in liver⁽²⁷⁾. Also bacterial products LPS and pro inflammatory cytokines IL1, IL6 and TNF both inducer for serum associated amyloid in hepatocytes and in activated macrophages and reticuloendothelial cells of spleen⁽²⁸⁾ which more observed in the immunization group 2 of mice in this study especially at 21th days post challenge with EPEC. Serum associated amyloid stimulate the cytokines IL12 for modulation of lymphocytes function in CMI⁽²⁹⁾ and it stimulate IL23 for recruitment

of active inflammatory cells responsible for the chronic inflammation and granuloma⁽³⁰⁾.

CONCLUSION

- 1-Immunization group 2 of white mice with whole sonicated EPEC Ag and BCG vaccine give high level of CMI and HI with elevation of IgG, IgM comparable to group 1 immunized with whole sonicated EPEC Ag alone.
- 2-Mild bacterial isolates seen in internal organs of immunized group 2 with whole sonicated EPEC Ag and BCG vaccine comparable to moderate bacterial isolates in whole sonicated EPEC Ag alone group 1.
- 3-More localized granulomas in internal organs of immunized group 2 with whole sonicated EPEC and BCG vaccine comparable to mild granulomas in immunized whole sonicated EPEC Ag alone group 1.

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