ISSN NO. 2320-5407

Journal homepage: http://www.journalijar.com

INTERNATIONAL JOURNAL OF ADVANCED RESEARCH

RESEARCH ARTICLE

Study on protection role of BCG Vaccine in Immunization against Enteropathogenic Escherichia coli and there challenge Infection and Pathology in white albino mice

Dr. Khalil H.Al-Jeboori and Marwa S. Kezar

Dept. of pathology , College of Veterinary Medicine , Univ. of Baghdad , Iraq .

Manuscript Info

Manuscript History:

Received: 12 February 2014
Final Accepted: 22 March 2014
Published Online: April 2014

Key words:
Role of BCG in immunization & protection against EPEC 0119 challenge infection and pathology

Corresponding Author

Dr. Khalil H.Al-Jeboori

Abstract

This study aimed to determine the role of BCG in immunization of white mice against EnteropathogenicEscherichia coli (EPEC) and there role against challenge infection and pathology with this microbial agent . EPEC was isolated from diarrhea ofchildren , routinely diagnosed and serotyping were identified as 0119 EPEC . Then three groups of mice (30 each group) were taken , first group immunized with whole killed EPEC Ag, the second group were immunized with whole killed EPEC AgandBCG 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 28^{th} days post immunization , increase in DTH- skin test thickness at 24, 48 hrs in group immunized with whole killed EPEC Ag and BCG (group2) comparable to group 1 (immunized with whole killed cell Ag alone and group 3 (control group) . Also humeral immune (HI) response elevated in group 2 comparable to group 1 and control group3 . 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 compable to control group 3 following challenge dose infection with EPEC. Also more localized granulomatous lesion were seen in internal organs specially at 14^{th}. , 21th days post inoculation of challenge dose in group 2 comparable to group 1 and no lesion in control group 3.

Conclusion :

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

.

Introduction

EnteropathogenicEscherichia 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 that 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 diarreagenic role in dogs [5]. Bacillus CalamiteGuerin (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 killed 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 and Methods

Three groups of albino white mice were used in this study .

First group : immunized at first day and 14th day with whole killed EPEC 0119 Ag (0.1ml) S/c prepared according to [7] .

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

3rd group : 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 1gM and 1gG 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 $10x9^{10}$CFU/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 and Discussion

Delayed type Hypersensitivity (DTH) skin test :

The results showed increase thickness of foot bad of mice (1.71∓ 0.01 , 1.86 ∓ 0.06) at 24 hrs and 48 hrs respectively in groups 2 (immunized with whole killed EPEC Ag and BCG Vaccine) comparable to group 1 (immunized whit whole killed Ag alone , 1.67 ∓ 0.04 , 1.75 ∓ 0.05) at 24, 48 hrs. respectively and no (Negative skin thickness in control group 3) (Table -3)

Indirect Hemagglutination test (1HA):

The results showed increase in antibodies titer (102 ∓ 0.06) in group (2) of mice immunized with whole killed EPEC Ag and BCG comparable to group (1) immunized with EPEC Ag alone (64 ∓0.05) 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 killed EPEC Ag and BCG vaccine , the levels were 21.7 ∓ 0.17 for IgG and 43.64 ∓ 0.04 for IgM comparable to the level of IgG and IgM 18.32 ∓ 0.18 and 18.75 ∓ 0.14 respectively in group 1 immunized with whole killed 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)

Immunological Tests	Whole killed EPEC Ag		Whole killed EPEC Ag & BCG Vaccine		Control (pbs)	
DTH – skin test	Thickness of foot pad(mm)		Thickness of foot pad(mm)		Thickness of foot pad(mm)	
	24hrs	48hrs	24hrs	48hrs	24hrs	48hrs
	1.67∓ 0.04	1.75 ∓ 0.05	1.71 ∓ 0.01	1.86 ∓ 0.06	0	0
Abs titer IHA Test	64 ∓ 0.05		102 ∓ 0.06		4 ∓ 0.2	
Immunoelectorphoresis , level of IgG	18.32 ∓ 0.18		21.7∓ 0.17		9.55 ∓ 0.03	
Immunoelectophoesis , level of	18.75 ∓ 0.14		43.64 ∓ 0.04		9.43 ∓ 0.02	

IgM			

Table -1 :DTH – skin test , Antibodies titer and serum immunoelectrophoresis for IgGandIgM in groups of mice immunized with whole killed EPEC Ag & BCG and control .

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 induration 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 killed EPEC Ag and BCG vaccine) comparable to group 1 (immunized with whole killed 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 $1L_1$ from sensitized macrophages and $1L_2$ 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 killed EPEC Ag alone) .

The results showed high level of antibodies detected by IHA test and the type of Abs were 1gG and 1gM detected by the electrophoresis in group 2 (immunized with whole killed EPEC Ag and BCG vaccine) comparable to low level in group 1(immunized with whole killed EPEC Ag alone) . These results belong to the stimulation and potentiation effect of BCG with whole killed Ag comparable to whole killed 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 IgMthrough release 1L4 , 1L5 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 1L4 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 killed 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 group2 comparable to group1.

Clinical and Bacteriological isolation :

Both groups of mice (1,2,3) showed healthy animals during the course of experiment , the group2 (immunized with whole killed EPEC Ag and BCG vaccine) give protection rate 100% comparable to group 1 (immunized with whole killed 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 group2(immunized with whole killed EPEC Ag and BCG Vaccine) comparable to moderate bacterial isolates in group1 (immunized with whole killed EPEC Ag alone) and control group 3 , Pbs . (Table-2)

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

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

Note: + mild bacterial isolates

++ moderate bacterial isolates

_ No bacterial isolates .

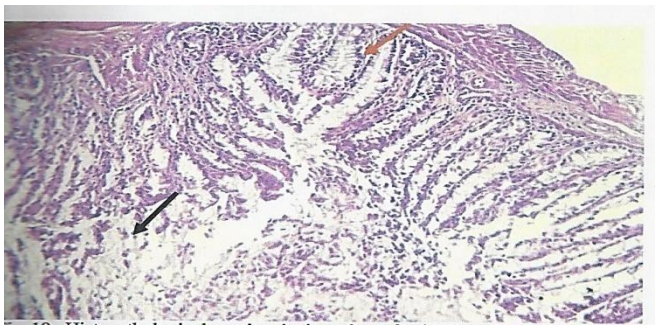
The mild bacterial isolates indicated that some bacterial colonies spread from the injection site into the internal organs such as livers, spleen , lung , kidney & intestine , these organs were enriched with macrophages which activated under effect of immunization with whole killed EPEC Ag BCG vaccine , the activated macrophages under the effect of interferon gamma produced by natural killer cells [18] in immunized group2 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 killed 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 1L12 both affecton natural killer cells to induce INF - ∂ 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 were seen in immunized group except hyperplasia of spleen in goup1, 2 of immunization .

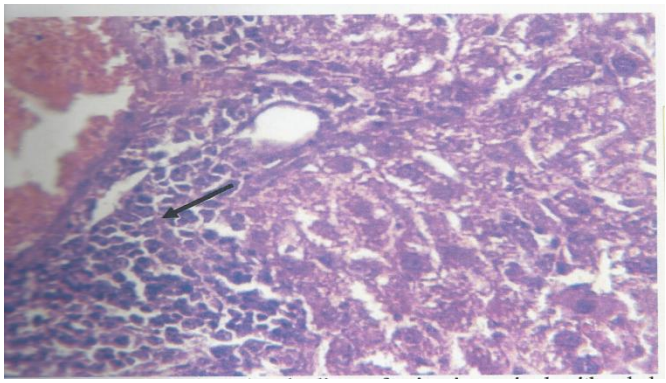
Microscopically :

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)



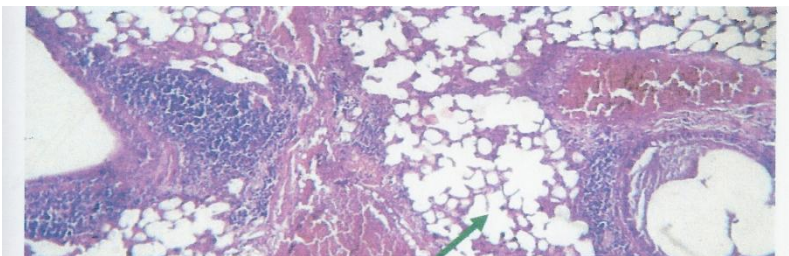
(Fig- 1) : intestine showed hyperplasia of goblet cells and mucinous degeneration (H&E)x40 .

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



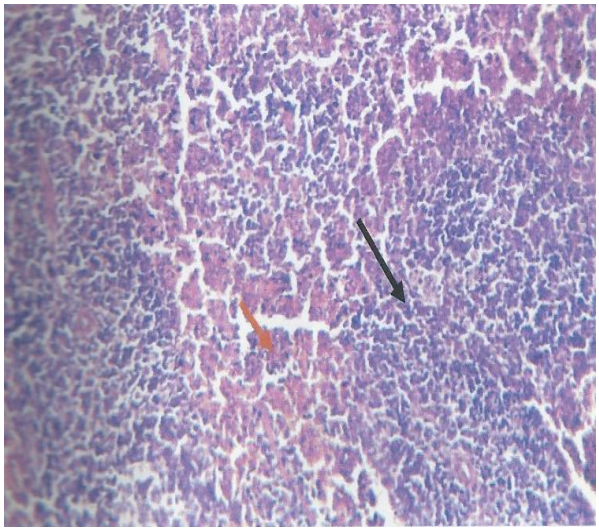
(Fig-2): Liver showed early granuloma and congestion of central vein (H&E)x400

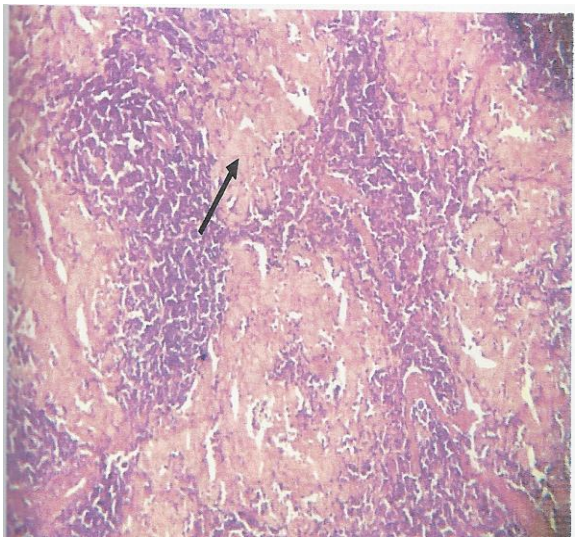
Lungs : showed extensive hyperplasia of peribronchial associated lymphoid tissue and congestion of blood vessels , emphysema and mononuclear cells infiltration in alveolar walls and adjacent to B.V. (Fig-3)



(Fig-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)

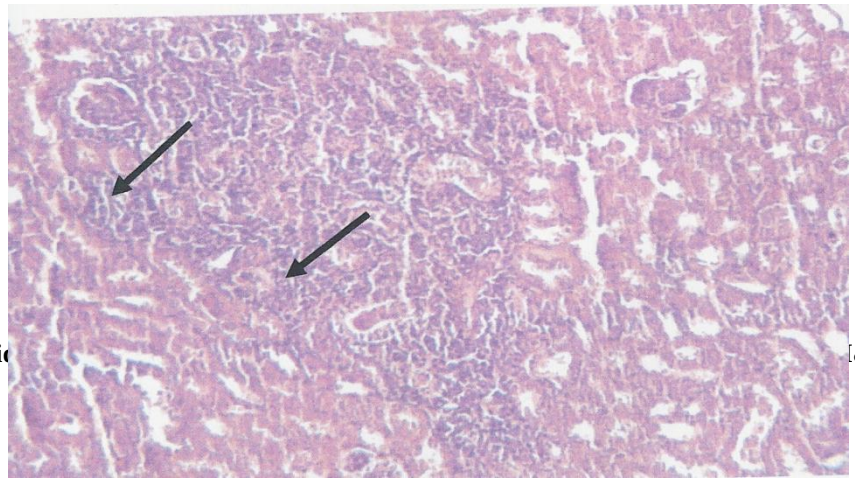




(Fig-4) : Spleen showed reactive hyperplasia of white pulp. (H&E)x40

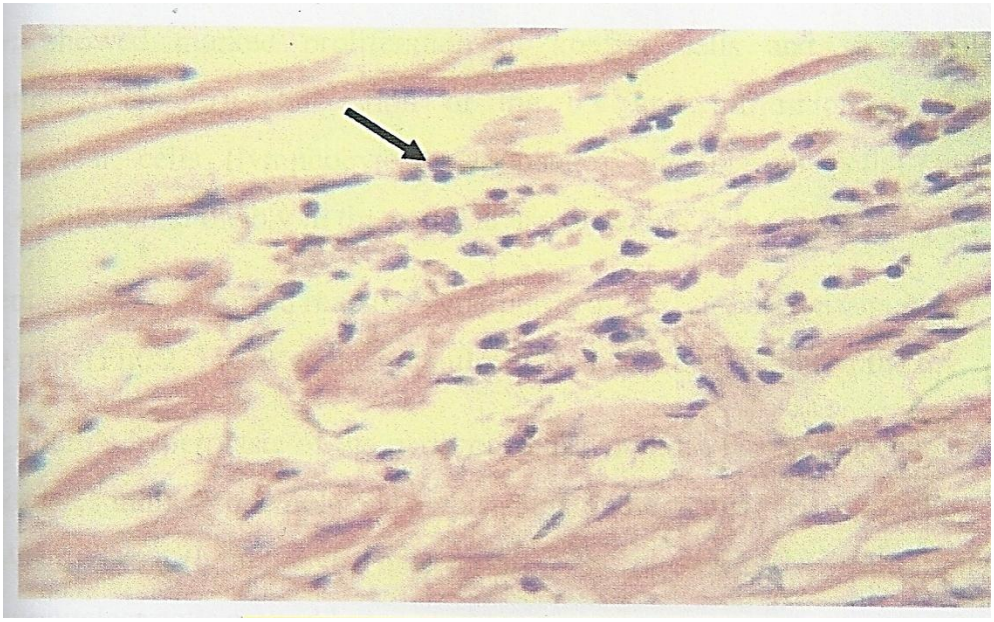
(Fig-5) : Spleen showed diffuse amyloidosis (H&E)x40

Kidneys : showed extensive cells infiltration of mononuclear cells and plasma in the interstitial renal tissue and



(Fig-6) :Ki &E)x40

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



(Fig-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 killed EPEC Ag and BCG vaccine , even Ag alone . BCG increase the number of phagocytes [22] which more evident in group 2 (immunized with whole killed 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 1L1, 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 1L12 for modulation of lymphocytes function in CMI [29] and it stimulate 1L23 for recruitment of active inflammatory cells responsible for the chronic inflammation and granuloma [30] .

The References :

1) Black , R. F. and Brown , K. H (1982) .Longitudinal studies of infectious diseases and physical growth of children in rural Bangladesh . Incadence of diarrhea as association with known pathogens. Am. J. Epidemiol. 115: 315-324 .
2) Blanco , J. E; Blanco , M; Blanco, J; Mora, A.; Balaguer , L. ; Mourino , M. ; Juarez, A. and Jansen , W. H. (1996) O serogroups , biotypes and eae genes in Escherichia coli strains Isolated from diarrheic and healthy rabbits J. clin . Microbiol 34: 3101-3107 .

3) Fischer, J; Maddox, C; Moxley , R. Kinden , D and Miller , M. (1994) . Pathogenicity of a bovine attaching effacing Escherichia coli isolate lacking shiga- like toxins. Am. J. Vet. Res. 55: 991-999 .
4) Zhu, C. ; Harel , J.; Jacques , M. ; Desautes , C; Donnenberg , M. S; Beaudry , M and Fairbrother , J. M (1994) Virulence properties and attaching effacing activity of Escherichia coli 045 from swine postweaning diarrhea Infect. Immun 62: 4153 -4159.
5) Foster, G. ; Ross , H. M; pennycott, T. W. ; Hophins , G. F and Mc Laren , I. M (1998) . Isolation of Escherichia coli 086 –K61 producing cytolethal distending toxin from wild birds of the finch family .Lett, APPL. Microbiol. 26: 395-398 .
6) Bannon , M. J. (1999) BCG and tuberculosis Arch. DiS. Child 80: 80-83 .
7) Dhasarathan , P. ; Sarvanan , S. and perianayagasamy , A. (2010) Disease management by vaccination susingEscherichia coliantigens . Afr. J. Pharmacol. 4(3): 119-122.
8) Herbert, W. J. (1978) passive hemagglutination with special reference with tanned cell techniques cellular immunology ch. 20 in : Weir. D. M. Handbook of experimental immunology 3rd. ed. Black well scientific publication 11(20) : 1-20 .
9) Hay, F. C. and westood, O. M(2002) serum preparation of immunoglobulin in practical immunology 4thed. Blackwell science .
10) Dixon , W. J. (1980) Efficient analysis of experimental observations . Ann. Res. Pharmacol. Toxicol. 20: 441-462 .
11) Luna, L. G(1968) Manual of histological staining methods of the Armed Forces Institute of pathology ,3rd . ed .McGraw –Hill Book company New York .
12) Tizard , I. R. (1992) VetrerinvyImmunology W. B. Saunders – company , Philadelphia , Pennsylvania , USA .
13) Chow, Y. H; Ciang , B. L. Lee, Y. L. ; chi, W. K; Lin , W. C; Chen , Y. T. and Tao, M. H. (1998) . Development of Th1 , Th2 population and the nature of the immune response to hepatitis B virus DNA vaccine can be modulated by co-delivered of vaccine , cytokine gene . J. Immunol. 60: 1320-1329 .
14) Yilu, X. C. , Tracy , M. D; William , C. D. and Michael, A. O (1999) INF - γ 2B enhance Th1 response in bladder cancer patient receiving Mycobacterium bovies BacillusCalmette Guerin Immunotherapy . J. Immunol. 162: 2399-2405 .
15) Katina , M. G. ; Hideho, O; Cynthia , S. Brissetle , S. L. and Villa, J. A(2000) Cytokine gene therapy of Gliomas : Induction of ($CD4^+$ T cells) by interleukin – 4 – transfected . Cancer Res. 60: 2449 – 2457 .
16) Aliabbas , A. H.; Rajpal , S. K; Devanand , R. K. ; Shubang , R. and Hemant, J. P. (2010) effect of BCG in vaccination on humeral response in mice model. Ind. J . Exp. Biol. 49: 7-10 .
17) Hsieh , C. S; Macantonia , S. E; Tripp, S. Ogrra, A and Muphy, K. M (1993) development of Th1 $CD4^+$ T cell through 1L12 produced by Listeria – induced macrophages . Science 260: 547 – 549 .
18) Baily , G. V(1980) the efficacy of BCG vaccination – A brief report of the chingleput BCG trial . NTI news lett. 17: 108 .
19) Rotroson , D. and Gallin, J . (1987) Disorders of phagocytic function . Ann. Rev. Immunol. 6: 12- 50 .
20) Katoch, K. (1996) Immunotherapy of leprosy. Ind. J. Lepr. 68(4): 349-361 .
21) Collines, H. I; Ulrich , E. S and Stefan, H. E. (1998) . Early 1L4 indction in bone marrow lymphoid precursor cells by mycobacterial lipoarabinomannon . J. Immunol. 161(10): 5546- 5554 .
22) Conti, B.; Jahng, H. W; Tint , C. I ; Son , J. H. and Joh, T. H. (1997) induction of interferon – gamma inducing factor in the adrenal cortex. J. Biol. Chem. 272: 2035 – 2037 .
23) Schlech, W. F. (1996) . Pathology and immunology of L. monocytogenespathol. Biol. Press 44: 775-782 .
24) Roderick, N. (2010) . Medical microbiology , 25th . ed pp. 121-130 .
25) Saches . L. and Lotem. J. (2004) Thenetweork of hematopoietic cytokines. Proc. Soc . Exp. Biol . Med 1206 : 1170 – 1175 .
26) Uhlar, C. M and Whithead, S. (1999) Serum amyloid A, the major vertebrate acute phase reactant . Eur. J. Biochem. 265: 501 -523 .
27) Jensen , L. E. and whithead, A. S. (1998) Regulation of serum amyloid A protein expression during the acute phase response . Biochem . J. 334: 489 – 503 .
28) McCormic , D. B. (2004) Two interconnected B – vitamins : Riboflavin and pyridoxine . Physiol. Rev . 69: 1170 – 1198 .
29) Rong; H. E; Larry , W. and Shepard, J . (2006) serum amyloid A is an endogenous ligand that differently induced 1L12 and 1L23 . Immunol . J. 177: 4072 – 4097 .
30) Laungrish , C. L.; McKenzie , N. J. and Wilsin , R. (2004) 1L12 and 1L23 master regulators of innate and adptive immunity . Immunol . Rev. 202: 96-105 .