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EXPERIMENTAL CHRONIC TUBERCULO-TYPHOID EXPOSURE IN A LAPIAN IMMUNE EVALUATION MODEL II: CUMULATED EFFECT

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ABSTRACT

Aim of the Study: To evaluate the possible influences of chronic cumulated exposure to lower concentrations of tuberculin on the immune response to typhoid antigen in a lapin model. **Methods:** Residual tuberculin concentration of 0.00005 ,0.0005, 0.005 and 0.05 IU were nasally applied through aerosolizer in a week wise manner to ten newzeland white rabbits, five of which were left four days then immunized with *S. typhi* "O" antigen (group I). The other five however were left as such for one more week (Group II). Ten other neuzland white rabbits were equally subdivided in to *S. typhi* immunized rabbits (Group III) and saline injected rabbits as control (Group IV). A set of immune function test were performed to the four groups of these rabbits. **Result:** The cumulated tuberculin exposure (Group II) lead to increase of NBT neutrophil phagocytosis ; significant LIF percentage; E.rossete count; IL-1α, IL-12 and IL-10 but not IL-4 and skin DTH. While combined Tuberculo-Typhoid exposure (Group I) lead to increase in NBT, E.rosette and IgM anti S. typhi "O" but not DTH. Both of the exposure modalities shifts cytokine balance towards proinflammatory cytokines. **Conclusion:** The major immune feature of these exposure modalities was: 1- Immunomodulation, 2-T-Cell competition, 3-Cytokine unbalance and 4- inhibition of IgM class switching to IgG.

KEY WORDS: Experimental Chronic, Tuberculo-Typhoid Exposure, lapian Evaluation Model.

INTRODUCTION

Chronic intracellular bacterial infections may release residiual bacterial proteins within the vertebrate host like man. Such release can be immunotoxic to the host [1]-[5]. In the present work residual tuberculin concentration increasing up to 0.05 IU were nasally applied through aerosolizer followed by four days relief then immunized with two oral then after two intravenous doses of *S. typhi* in a lapian model.

MATERIALS AND METHODS

1-Tuberculin

Tubersol is a sterile isotonic solution of Tuberculin (Purified protein derivative of *M. tuberculosis* (5TU per 0.1 ml) in phosphate buffered saline containing Tween 80 (0.0005%) as a stabilizer. Phenol 0.28% is added as a preservative [6], [7]. Four sequenced concentrations (0.05TU, 0.005TU, 0.0005 TU& 0.00005TU) were made in phosphate buffer saline.

2-*S. typhi* "O"

antigen (bacterin) as heat killed antigen was prepared as [8].

3-*S.typhi* Sensitine

Sensitine is cell free culture filtrate. It was prepared as 24hr culture in 0.5 % glucose peptone water. Growth centrifuged at 2500 rpm for 5 min. Supernatant was filtrated through 0.22 mm. membrane filter. [9].

4-Rabbits

Twenty male newzland rabbits were of 1-1.5 kg body weight kept a libitum and housed in individual wire-rod floored and stain-less steal cages, each measuring 48 x 16 x 46 cm with collection Pan beneath each gage. They were grouped into four groups each of five as in the follow diagram Fig-1.

5-Tuberculin Inhalation Technique

Inhalation was performed using compressor nebulizer (Mabie Co .England). It passes aerosol containing tuberculin of 0.5 to one mm size for easy inhalation.

6- *S.typhi* Immunization

The immunization program was performed as in flow fig.(1).

7- Blood Sampling

From each animal in each group 10 ml blood through cardiac puncture. Five with anticoagulant and five without anticoagulant. Those with anticoagulant was for cellular immune function, while, those without was fzor serology tests.

8-Immune Function Tests

Various immune function tests were done as in Steven 2010. Cytokine determination was performed as in manufacture instructions.

Group I	Group II	Group III	Group IV
Four sequential increasing tuberculin concentrations as 1st week 0.00005 IU 2nd week 0.0005 IU 3rd week 0.005 IU 4th week 0.05 IU Leave four days then the rabbits receives two weekly oral doses and two weekly oral doses and two weekly intravenous doses of *S.typhi* O vaccine leave one week,bleed	Four sequential increasing tuberculin concentrations to the same rabbit groups 1st week 0.00005 IU 2nd week 0.0005 IU 3rd week 0.005 IU 4th week 0.05 IU leave one week then bleed	*S.typhi* control 5 rabbits 1st and 2nd weeks oral 3rd and 4th weeks intravenously ,one week leave then bleed.	Saline control received saline as in III programe.

Fig.(1) Immunization protocol

RESULTS

Cellular Immune Function (Table 1)

Nitroblue Tetrazolium Reduction NBT

The percentage of NBT phagocytosis were 43,58,63 and 31%for the groups I,II,III and IV respectively.

Leukocyte Inhibitory Factors(LIF)

LIF percentage were showing 74; 41.5; 52.03 and 98.8 for the groups I,II,III and IV respectively.

Erosette Count

The E.Rosette T-cell counts were showing percentage of 72; 71.5; 71.6 and 27.5 for the groups I,II,III and IV respectively.

III- Delayed Type Hypersensitivity skin test (table-3):

Using tuberculin and *S.typhi* cell free culture filtrate(CFCF) as skin sensitizer through ID rout to rabbits group I,II,III and IV. It was evident that four scores were noted as typical tuberculin , tuberculin type, Jones Mote Reaction and anergy.The induration area were 5,6,17 and 0 mm for the groups I,II,III and IV respectively.

IV- Humoral antibody responses (Table-4):

The anti *S. typhi* "O" agglutinin titers were showing 96,20,384 and 20 for the groups I,II,III and IV. The concentration of *S.typhi* IgM were 0.0816, 0, 0.512 and 0.073 while for *S. typhi* "O" IgG were 0.0298, 0, 0.231&0.017 for the groups I,II,III and IV respectively. IgM were of higher concentration than IgG in the groups I,III and IV respectively. Antituberculin antibody titer were showing 0, 80,0 and 0 in the groups I,II,III and IV respectively.

Table-1:Cellular Immune Feature of test and control rabbits.

Feature	Test		Controls	
	Tu+*S. typhi*	Tu	*S.typhi*	saline
NBT	43	58	63	31
LIF	74.5	61.5	52.03	98.8
E.Rosette	72	71.5	71.6	27.5

Table-2: Cytokine profileand Cytokine unbalance

Cytokine	Test		Control	
	Tu+*S. typhi*	Tu	*S.typhi*	saline
A-Profile				
IL-1α	91.33	220.477	56.69	15.3
IL-12	60.454	24.477	24.56	7.32
IL-10	15.374	21.145	18.8	4.5
IL-4	2.4	1.811	0.07	99.1
B-Cytokine unbalance				
Groups	Proinflammatory		Antinflammotory	
	IL-1α	IL-12	IL-4	IL-10
Tu+*S. typhi*	91.33	5.133	2.7	15.374
Tu	220.44	74.477	1.811	21.147
S.typhi	56.64	24.56	0.017	18.8
saline	75.3	7.32	99.1	4.5

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Table-3: Delayed hypersensitivity Skin Test in Test and Control Rabbits.

Group	Erythema 24-48hr		Induration 48-96hr		Jones Mote Reaction	
	CFCF	Tu	CFCF	Tu	CFCF	Tu
Tu+ *S. typhi*	++	+/++	6	5	1/5	2/5
Tu	+	0	0	0	-	0
S. typhi	++\+++	-/-	0	17	0	1/2
Saline	-	-	-	-	-	-

Table-4: Humoral Antibody Responses of Test and Control Rabbits

Type	Titer			
	Tu+ *S. typhi*	Tu	*S. typhi*	Saline
Anti- *S. typhi*	96	20	384	
Antituberculin	0	80	0	0
IgM. anti*S. typhi*	0.0816	0	0.5125	0.073
IgG anti *S. typhi*	0.0298	0	0.231	0.017

DISCUSSION

The NBT neutrophil phagocytosis in the test and *S.typhi* control were higher than the saline control (Table-1). Group-1 although was showing increase as compared to saline control but it was lower than group II and III. There may be a competing epitope in *S.typhi* that might suppress NBT phagocytosis**.** LIF in group I and IV was non-significant but group I was around 70% and group II Tu alone poses significant low 40.5% LIF. Thus an epitope from *S.typhi* may inhibit LIF cytokine production in group I [10], [11]. CD2 is documented in rabbit lymphocyte of T-cell subsets. Control, saline showed 27.5% the other groups were showing counts around 70%, this mean that *S. typhi* , Tuberculin+ *S. typhi* and Tuberuculin Induce increase in T cell counts [12]-[15].The cytokine IL-4 was suppressed by treatment with *S. typhi*, Tuberculin+ *S. typhi* while tuberculin increase IL-1α, IL-12 and IL-10 in group II and to lesser extend in group I[16]. IL-1α, IL-12 as proinflamatory cytokines were highly exceeding IL-10 and IL-4 as anti-inflammatory cytokines a situation that present a case of cytokine unbalance (Fig-2).Single 1 IU in a dose of 0.1 ID yield 13-15mm induration area in tuberculin treatment rabbits one week post injection [17].

Chronic exposure to increasing low doses of tuberculin (Table-3) suppress the induration areas in treated rabbits group I. to the mean of 5 mm and to nil in group I. Thus such chronic exposure can be skin DTH suppressor, T cell competition (Tu+*S.typhi*) [18] or it present a case of low dose cellular tolerance[17],[19].

Jones Mott reaction was noted in 1/5, 2/5 of animal in group I and III but not in group II. With CFCF and Tuberculin as sensitizer in group I and III respectively [20]. Group I was showing low anti *S. typhi* "O" antibody titer as compared to group III. This can be due to competitor epitope in tuberculin that suppresses *S. typhi* "O" agglutinin titer[21]. *S. typhi*IgM index were far more than IgG in group I, and higher than group III and IV. Chronic exposure to tuberculin at low concentration inhibit class switching to IgG [2]. The cumulate tuberculin chronic exposure lead to increase of NBT, LIF, E.rosette, IL-1α, IL-12,IL-10 but not IL-4 while combine Tu+*S. typhi* exposure lead to NBT, E.rosette and IgM anti *S. typhi* . Both exposure modalities shift cytokine balance towards proinflamatory cytokines [22]. Therefore, tuberculin and tuberculo-typhoid exposure modulate rabbit immune system towards immunstimulation rather than immunsuppresion. (Table 1-4). Humoral and cellular antigenic competition were noted in *S. typhi* agglutinin and DTH induration respectively (table 3 and 4). Shift toward proinflamatory cytokines were noted (table 2) and inhibition of class switching from IgM to IgG (table 4).

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