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**Research Article**

## Lapin cryoglobulin responses to *Brucella melitensis*

### RV-1

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#### **ABSTRACT**

Natural human brucellosis has been documented to be associated with secondary cryoglobulinemia in this area. An attempt was made to use *Brucella* vaccine, for the induction of secondary cryoglobulinemia in a laboratory animal model. The test immune system was that of rabbit, the stimulating agent was *Brucella melitensis* Rev-1, Lio-Vac. Vaccine and the immunization protocols were nasal mucosal (Group I), Intramuscular-Subcutaneous (Group II) and Intramuscular-Intra-venous (Group III). The adopted cryoglobulin inclusive criteria were; Reverse precipitation at 4°C, dissolution at 37°C or at 45°C, protein in nature and reacts positively with *Brucella* vaccine. The immunization protocols have shown variable cryoglobulin responses. Group III has shown higher cryoglobulin response in term of concentration followed by Group II and lower cryoglobulin concentration. Secondary immune responses have shown higher responses than primary immune responses among the three test groups. Thus, from these findings one may conclude; i- An experimental lapin model for secondary *Brucella* cryoglobulinemia was developed. ii- The facultative intracellular (*Brucella*) was just like obliged (*Mycobacterium tuberculosis*) intracellular bacterial pathogens in ability to induce secondary cryoglobulinemia in the lapin models. iii- The nature of the immunization protocol effect the degree of cryoglobulin responses in term of concentrations and iv- The more the exposure to *Brucella* vaccine, with in the used vaccine dosage limits the more the cryoglobulin response.

**Keywords:** Natural human brucellosis, *Brucella melitensis* vaccine RV-1 and cryoglobulin response.

#### **INTRODUCTION**

The *Streptococcus pneumoniae* polyvalent vaccine had been applied for the immunization of rabbits, sera obtained from the vaccine primed rabbits have shown presence of large amounts cryoglobulin in their sera<sup>1</sup>. *Streptococcus* group B has been tried in immunization of rabbits the protocol covers both of the primary and secondary immune responses. Secondary immune responses have shown an earlier and higher response in term of concentration of the resulted cryoglobulin than the primary immune responses<sup>2</sup>. BCG has been found to be marked inducer of cryoglobulin responses both in primary and secondary immune responses, though the secondary was higher than the primary<sup>3</sup>. The somatic "O" antigen of *Salmonella typhi* tried in three different immunization protocols in rabbits. It has been evident that *S. typhi* cryoglobulin responses in rabbits using the three different protocols were of

comparable cryoglobulin concentrations<sup>4</sup>. The present work *Brucella melitensis* vaccine was attempted as a stimulant for cryoglobulin responses in rabbits.

#### **MATERIALS AND METHODS**

##### **1. Cryoglobulin Stimulant:**

*Brucella melitensis* Rev-1, Lio-vac. (C.Z. Veterinaria, Spain Ministry of Agriculture). To be used as stimulant, it was diluted to have  $1 \times 10^6$  vaccine unit /ml.

##### **2. Lapin Immunization Protocols<sup>5</sup>:**

Twelve Chinchilla rabbits were adapted to housing conditions for two weeks and kept at Libidum during experimentation period. Handling and care of rabbits was in accordance with the guidelines for research on rabbit implemented by the international council of laboratory animal science. Rabbits were

subdivided into four groups each of three, Table 1 and assigned I to III as in the following:

- Nasal mucosal.....group I
- Intramuscular subcutaneous group II
- Intramuscular-Intravenous... group III
- Control, received saline..... group IV

**3. Cryoglobulin Response:**

The inclusive criteria for the presence of cryoglobulin response were; Precipitate nature at 4 °C, dissolve at 37°C or 45C as well as reprecipitate at

4°C, significant cryocrit %, measuring protein concentration, has anti-Brucella immune specificity together with positive and negative controls<sup>6</sup>.

**4. Biometry:**

The group, each group mean, variance, standard error, standard as well as the two tailed “t” test for the differences between the group means were calculated in accordance with Steel et al<sup>7</sup>.

**Table 1**  
**The Cryoglobulin Immunization protocols in rabbit<sup>[5]</sup>**

Features	Nasal group I	(f)IM – (g)SC group II	IM – IV group III
First week Then;  Test for cryoglobulin	One ml. drop-wise in both nostrils	1/4 ml ,LIM(a) 1/4 ml ,RIM(b) 1/4 ml, LSC(c) 1/4 ml. RSC(d)	1/2 ml, IV(c) 1/4 ml, LIM 1/4 ml, RIM
Second week	One ml. drop-wise in both nostrils	As in first week	As in first week
Third  Fourth week Then, Sixth week then	One ml drop wise in both nostrils Leave Test for cryoglobulin Leave Test for cryoglobulin	As in first week  Leave Test for cryoglobulin Leave Test for cryoglobulin	As in first week  Leave Test for cryoglobulin Leave Test for cryoglobulin

(f)IM=Intramuscular, (a)LIM=Left intramuscular ,(b)RIM=Right intramuscular  
(g)SC= Subcutaneous , ©LSC = Left subcutaneous,(d) RSC = Right subcutaneous  
(e)IV = Intravenous

**Table 2**  
**Biometry of the Brucella vaccine induced cryoglobulin response using nasal immunization.**

Test Animal	CryoglobulinPrimary First week; mg/ml	CryoglobulinSecodary Fourth week; mg/ml	CryoglobulinSecondary Sixth week; mg/ml
Findings;R1 R2 R3	3.8 2.7 5.3	8.1 7.2 9.98	8.0 2.6 9.5
Statistics Low limits Means High limits Range SE SD	2.7 3.93 5.3 2.6 1.028 1.772	7.2 8.36 9.98 2.6 2.387 4.135	2.6 6.7 6.7 6.9
"t" S-P,2+2	3.5669		
Table"t" atP .95	3.66		

S=Secondary P=Primary

**Table 3**  
**Biometry for Brucella vaccine induced cryoglobulin responses using intramuscular-subcutaneous immunization protocol in rabbit.**

Test Animals	Primary Cryoglobulin, 1 <sup>st</sup> week, mg/ml.	Secondary Cryoglobulin, 4 <sup>th</sup> week, mg/ml.	Secondary Cryoglobulin, 6 <sup>th</sup> week, mg/ml.
Findings, R1	5.2	15.6	13.8
R2	6.3	18.6	18.8
R3	9.7	23.8	23.3
Statistics			
Low limits	5.2	15.6	13.8
Mean	7.66	19.366	18.366
Higher limits	9.7	23.8	23.3
Range	4.5	8.2	9.5
SE	1.028	4.387	
SD	1.772	4.135	
"t"			
S-P	4.1713		
2+2			
Table "t" at P.95	3.66		

**Table 4**  
**The Biometry for the Brucella vaccine induced cryoglobulin responses using intramuscular-Intravenous rout in rabbit.**

Test Animals	Primary Cryoglobulin, 1 <sup>st</sup> week, mg/mh.	Secondary Cryoglobulin, 4 <sup>th</sup> week, mg/ml.	Secondary Cryoglobulin, 6 <sup>th</sup> week, mg/ml.
Findings R1	8.3	23.0	15.3
R2	11.5	26.5	26.0
R3	13.6	21.3	25.4
Statistics			
Low limits	8.3	21.3	15.7
Mean	11.8	28.6	23.33
Higher Limits	13.3	26.5	25.4
SE	1.705	1.673	
SD	2.945	2.497	
"t"			
S-P	7.033		
2+2			
Table "t" at P.95	3.66		

## RESULTS

The Brucella primed rabbits sera were found containing two types of globulins as nomoglobulin and cryoglobulin. Both rabbit primed sera and cryoprotein fraction were positive when reacted with B. melitensis vaccine. The cryoglobulin was found, precipitable at 4°C and dissolvable at either or both of 37°C, 45°C and reprecipitable at 4°C. Thus Brucella Cryoglobulin responses were evident using nasal, intramuscular-subcutaneous, intramuscular-Intravenous immunization protocols in rabbit. The cryoglobulin responses mimicked normo-globulin responses in being inclusive for primary and secondary immune responses. The secondary immune responses in term of concentration have shown doubled concentration means than that of primary

immune responses using the three immunization protocols (Groups I-III). The cryoglobulin concentration means for the groups; I, II, and III were; 6.330, 11.386 and 20.91 mg/ml respectively Tables 2 and 3. The best Brucella vaccine immunization protocol was that of intramuscular – intravenous followed by the intramuscular-subcutaneous. The lowest was that of nasal rout. The biometric analysis has shown that the cryoglobulin concentration mean differences between the secondary and the primary responses using two tailed t test to the groups I, II, and III were significant Tables 2, 3 and 4. The difference of the concentration means between the groups III and I and group III and II were significant, while to the differences between the groups II and I was not significant Table 5.

**Table 5**  
**The biometry for the intergroup mean differences to Brucella vaccine induced cryoglobulin responses in rabbit**

Immunization Protocol	Cryoglobulin group concentration,mg/ml.	Statistical significance
Control	-	
Group I	6.330	
Group II	110386	
Group III	20.91	
"t" II-I 2+2	2.4915	Nonsignificant
"t" III-I 2+2	7.6596	Significant
"t" III-II 2+2	7.8417	Significant
Table "t" at P.95	3.66	

## DISCUSSION

The induced brucella cryoglobulin responses in a lapin(Tables 2,3,4,5) model simulate that of natural human\* brucella infection associated cryoglobulin<sup>6</sup>. The developed brucella associated secondary cryoglobulinemia in the lapin model gave the evidence that brucella in natural infection of man and as a vaccine in rabbit model is implicated in initiation of cryoglobulin responses. Nasal mucosal surface have shown higher induction ability for tuberculus cryoglobulin and equivocal induction ability for typhoid cryoglobulin than that of brucella cryoglobulin taking in consideration that the three cases were in lapin models<sup>3-5</sup>. Brucella cryoacting epitope may be of non-intrinsic immunologic adjuvanicity and/or low immunogenicity<sup>8-10</sup>.Such epitope(s) charaters may stay behind the low brucella induce-ability to the nasal mucosal cryoglobulin response in comparison to M. tuberculosis and S. typhi<sup>11</sup>. Strict or facultative intracellular persistence may be a favorable condition for the bacterial pathogen to be an inducer for cryoglobulin responses<sup>12</sup>. Chronicity seems to be among the other required predisposing factors for initiation of cryoglobulin by bacterial pathogens.<sup>13-15</sup> The immune features of the brucella cryoglobulin responses may briefed as; i-Brucella meletensis may bear cryoacting epitope initiate secondary cryoglobulinemia both in man and rabbit.<sup>4,8</sup> .ii-Brucella cryo acting epitope can be T in-dependent , or dependent.<sup>16,17</sup> iii-Facultative intracellular existence and chronicity seemed to be pre-request for cryoglobulin response induction. iv-The more

brucella vaccine exposure the more cryoglobulin response initiated. And v-Rabbits cryoglobulin responses memicate that of normo-globulin response in being have primary and secondary response patterns. Secondary responses have higher concentrations than primary.<sup>2,3</sup>

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