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RESEARCH ARTICLE

THE IMMUNE FEATURES OF SALMONELLA TYPHI SOMAIC "O" ANTIGEN INDUCED LAPIN CRYOGLOBULINEMIA

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ABSTRACT

The present work was aimed at developing an animal model for the delineation of the immune features of the Salmonella typhi associated cryglobulinemia. The elected immune system was the rabbit. The test stimulant was S.typhi O antigen and the specific immune priming protocols were: Two systemic, one mucosal multisite, and one combined systemic-mucosal routs. The attitude was being through the demonstration for the effects of the time duration after the end of priming protocols. S.typhi O antigen can stimulate cryoglobulin responses using systemic, mucosal as well as combined systemic-mucosal routs. As the time duration post to the ends of priming protocols proceeds from 15 to 30 then to 45 days cryoglobulin response increased in terms of concentration, specific cryoglobulin antibody activity for typhoid O antigen as well as the shared human rheumatoid factor antibody acticvity. Bio meteric evaluation for the differences between the different tested priming protocols were non significant. However, such evaluation were significant when were done to the differences, between the time durations between 15,30 and45 days as well as 45 and 15, 30 and 15 and controls. Finally the major immune features of the typhoid lapin cryoglobulin were, cryoprotein, precipitable at 4C, dissolvable at 37C, reprecipitable at 4C and having specific antibody and shared autoantibody activity.

INTRODUCTION

The laboratory animal models that are simulating the natural occurring cryoglobulinemias in man were of several types (1-8). The stimulants were whole or parts of viruses,whole or parts of bacteria (Kowalewska *et al*., 2007; Nakashim and Kato, 1974; Herd, 1973). The attempted animal models have been; mice, transgenic mice, rat, and rabbit (Kowalewska *et al*., 2007; Serpertini *et al*., 1988; Castsoulis *et al*., 1965; Nakashima and Kato, 1974 and Herd, 1973). The immune priming protocols were divers. Among the tried stimulants were; Streptococcus, capsular materials of Klebsiella, BCG and tuberculin (Castsoulis *et al*., 1965; Nakashima and Kato, 1974; Herd, 1973; Shnawa and Jassim, 2011). The objective of the present work was to use S.typhi somatic O antigen as a stimulant for a lapin typhoid associated cryoglobulinemia and fixing the immune features of the mounted cryglobulin response.

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MATERIALS AND METHODS

A-The Antigen

S.typhi O antigen was prepared (Stevens, 2010) to the density of 9×10^8

B-Rabbits

Thirty-six rabbits of 1 to 1.5 kg. body weights whose proved to be free of ecto, endo and/haemo parasites as well as free of S. typhi O antibodies. These rabbits were grouped into four test and one control group. The test groups were each of eight, while, the control was four rabbits. These animals were adapted for the housing conditions for one week and kept at libidum conditions for food and drink with daily cleaning module.

C-Immune Primings

The antigen density was 9 /10 8/ml., dosage to test rabbit (ALShahery and Shnawa, 2006) groups I-IV as in the fallowing protocols;

Group I; Inta dermal (ID)- Subcutaneous (SC),received 1.2 ml as, 0.5 ml SC left (L), 0.5 ml SC right(R), 0.1 IDL, and0.1ml

ID in frequency of 3 injections in one week a part fallowed by one week leave……..…….……8 R

Group II; Intra venous(IV) – Intramuscular(IM) received 2 ml., as 1ml. IV, 0.5 ml.IML, 0.5 ml.IMR in the frequency of three injections in one week a part fallowed by one week leave……..……8R

Group III; Intra muscular – Subcutaneous received, 2ml., as 0,5ml.IML,0.5ml.IMR,0.5 ml.SCL, and 0.5 ml.SCR with a frequency of three injection in one week a part fallowed, by one week leave…….……8R

Group IV; Intranasal(IN) – Intra rectal (IR), received 2 ml as One ml IN and one ml.IR with frequency of three injection in one week a part fallowed by one week leave……..8R.

Control; Received normal saline in two ml. amounts as in group one…….…….5R., then at 15, 30 ,45 days post to the end of the priming protocols, blood samples were collected, sera obtained (Stevens, 2010) and cryglobuulin responses were assessed as in (Lynch, 2006).

D-Cryoglobulin Assessments;

The assessment of the cryoglobulin responses were carried out in accordance with the 14th standards re commended by Lynch *et al*. (2006).

E-Serology

Direct bacterial agglutination and semi-quantitative rheumatoid factor latex fixation were done as in (Stevens, 2010).

F-Biometery

Two tailed t test for the difference between means were performed as in (Steel, 1997).

RESULTS

A-Cryoprecipitates

Cryoprecipitates were apparent within one to four days incubation at 4C in the four test groups, however, one of the primed rabbits in the mucosal IN-IR gave cryoprecipitate in the second day Table 1.

Table 1. Cryocrit percentages assessed in the immune primed rabbit groups I – IV.

Days	I	II	III	IV
15,mean Mn	2.1	4.64	2.86	2.86
Median, Md	2	6.5	4	4
Range, R	2	5	4	4
30,Mn	4.6	7.7	4.71	4.76
Md	5	8	5	4
R	1.5	4	6	6
45,Mn	5.5	7.14	5.6	5.71
Md	5	7.5	3	5
R	1	2.5	4	4

Table 2. Cryoglobulin concentration in mg / ml. for the immune primed rabbit groups I – IV.

days	I	II	III	IV
Mean,15	16.32	18.44	18.06	18.51
30	20.72	21.72	20.01	21.08
45	21.36	22.22	20.5	22.19
Median,15	19	18.2	21	18.5
30	30	17.2	41.4	20.6
45	23	23	19.9	20.2
Range,15	10	6	9	2.1
30	6.3	7	6	7
45	4	5	7	6

Table 3. The ratios of cryglobulin to normoglobulin in the immune primed rabbit groups I – IV.

Days	I	II	III	IV
15	1: 2.3	1: 2.23	1 : 2.23	1 : 2.28
30	1 : 1.94	1 : 2.23	1 : 2.7	1 : 2.11
45	1 :2.16	1 : 2.3	1 : 2.19	1 : 2.13

Table 4. The shared lapin rheumatoid factors concentrations in mg/ml. for the rabbit immune primed groups 1 – IV with human rheumatoid factor.

Group/Days	Minimum	Mean	Maximum
I. 15	0	19.2	64
30	0	28.8	64
45	32	51.2	64
II.,15	0	25.1	64
.30	0	59.4	128
,45	16	61.7	128
III,15	0	13.7	64
,30	0	41.4	64
,45	0	42.4	64
IV,15	0	20.8	64
,30	0	32.1	64
,45	0	42	64

Table 5. Biometeric analysis for the concentration of the S .typhi O induced lapin cryoglobulin in the immune primed groups I – IV.

Statistical features	I	II	III	IV
S 2 1	18.72	8.648	8,76	48.435
S2 2	385.377	24.274	4.333	5.271
S2 3	2.915	2.501	5.386	4.648
SE1	1.938	1.1115	1.1118	2.657
SE2	8.47	1.862	0.786	0.815
SE3	0.764	0.5983	0.863	0.815
t 1,2	1.0066	1.4784	1.434	2.2480
t 1,3	2.005	3.1437	1.7341	2.3763
P level 1,2	NS	S,0.2	S,0.2	S,0.05
P level 1,3	S	S,0.01	S,0.1	S,0.05

The physics of the cryoprecipitates

The color of the precipitates were creamy or milky white and the texture was gelatineous.

C-Dissolving Temperature and Time

The cryoprecipitates dissolving temperature were 37C.While the dissolving times were ranged from 1/2 to2 1/2 hours Table 1.

D-Cryocrit

The four rabbits test groups were showing cryocrit percentages ranged from 1 – 8%. As the time post the end of priming protocols proceeds from 15 to 45 days, the cryocrit percentages were increased in the test rabbit groups Tables.

E-Cryoglobulin

Cryoglobulin was defined as the serum fraction that is precipitated at 4C, dissolved at 37C and re precipitated at 4C, biurt positive with concentration ranges from 16 to 23 mg/DL in various rabbits test groups. AS the time post the ends of priming protocols proceeds from 15 to 45 days, the concentration of the cryglobulin was increased.

F-The percentages of cryoglobulin to normoglobulin

Whatsoever .the time lasted from 15 to45 days with in the test rabbit groups the percentages of cryoglobulin to normoglobulin were around one to two.

G-Antibody Activity

Salmonella typhi O specific antibodies were detected in the rabbit test groups but not the control at the times 15, 30, and 45 days within a range of 80 to 100%.Group I was showing 4-5/5, group II 5-7/7, group III was 5-6/7 while ,group IV was with 6-7/7 positive for anti-S.typhi O antibodies in the tested cryoglobulin preparations groups I –IV.

H-Shared Human Rheumatoid Autoantibody Activity

There were immune priming method dependent variations in the extent of sharing between lapin rheumatoid autoantibody and human autoantibody rheumatoid factor .

I-Biometery

The concentration of cryoglobulins in the four test groups was elected to make the biometric analysis using concentration means, standard error and two tailed t test to the observations of 30 days to 15 days, 45 days to 15 days. Significant differences were found between those of 45 days to 15 days. In comparison the differences of the concentration means, SE and two tailed t test to the 45 days observation for the four priming methods were found Discussion. The natural infection induced cryoglobulinemias are mostly associated intracellular chronic protozoal, bacterial and/or viral pathogens. It had been reported in leprosy, tuberculosis, typhoid, brucellosis and viral hepatitis (Modi, 2000; Shnawa and ALgebori, 2012; Shnawa and ALgherani, 2014; Shnawa and Jassim, 2014; Shnawa and AlSerhan, 2014 and Gharagozloo *et al*., 2001). So far the laboratory animal simulation models are concerned, workers have been reported simulation models for virus hepatitis (4), glomerulo nephritis (20), vasculitis (Pastore *et al.*, 2001; Reugers *et al*., 2000), tuberculosis (Shnawa and Jassim, 2011), but not for typhoid, thus the present is being reported in a lapin simulation model using four immune-priming protocols .The findings presented in Tables 1-5 put forward an evidence of the action of S typhi O antigen as an inducer for typhoid associated secondary cryoglobulinemia. Two opinions have been stated to explain cryoglobulin. First, may be due to change in the intramolecular, intermolecular ionic bondings or change in isoelectric point of the globulin molecules (Jori *et al*., 1997and Ferri *et al.*, 2004). While the second holds that typhoid cryoglobulin may be secreted from a cryoacting B lymphocyte clones that grow, proliferate, expand and transform to plasma cell producing cryoglobulin (Castsoulis *et al*., 1965 and Shnawa and Jassim 2014). Rabbits cryoglobulins found to be immunopathogenic to allogenic rabbit producing hypersensitivity and autoimmunity (Shnawa *et al*., 2014).

The major immune features of such lapin croyglobulin responses were;

i. S.typhi O induced.
ii. Increased in the chronic course.
iii. Induced through systemic and mucosal routs.
iv. Different immune priming protocols showed comparable results
v. Have anti S. typhi O antibodies.
vi. Have shared human rheumatoid factor activity, such shared auto antibodies were increasing in concentrations as the time period after the end of the priming protocol increased
vii. Have comparative immune features to those induced by BCG and tuberculin (Shnawa and Jassim, 2011).

Conclusion

The lapin typhoid associated cyoglobulinemia was experimentally induced by S. typhi O antigen through systemic and/or mucosal routs .The response was of comparative features to that of BCG and tuberculin in rabbits.

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