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Proceedings of the 8th World Buffalo Congress

Caserta, October 19-22, 2007

Guest editors:

Rossella Di Paio (coordinator), Vittorio Baribieri Giuseppe Campanile,

Federico Infascelli, Vincenzo Piccolo

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Milano - Bolognaib)

Flow cytometry analysis of different T cell markers in mastitic buffalo milk before and after treatment with Homeopathic medicine

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ABSTRACT: The population of different T cell markers viz. $BoCD4^+$, $BoCDS^+$ and $BoWC1^+$ cells were analyzed using monoclonal antibodies (MAh) against T cell markers by Flowcytometric analysis There was significant increase in $BoCD4^+$ and $BoWC1^+$ T cells compared to healthy buffaloes (P<0.05). An increase was also recorded in $BoCD8^+$ cells of mastitic buffaloes as compared to normal buffaloes but this elevation was not significant (P>0.05) After the start of homeopathic treatment there was a gradual decrease in BoCDfP*, T

cells on day 5 and 10. However, on day 20 there was an increase in this cell subpopulation which was significant. There was significant increase in ratio of BoCD4+- BoCD8⁺ Thymphocytes on day 5 and day 10 post treatments indicating an up regulation of BoCD4⁺ On day 20 there was an apparent reduction in mean ratio of B0CD4⁺ BoCD8+ Tlymphocytes hut it remained more than one which is indicative of active immune response.

Keywords: Flow cytometry, BoCD4⁺ Lymphocyte, BoCD8 T Lymphocyte, Mastacure.

INTRODUCTION - In the present study, flowcytometric analysis of $BoC^{\circ}D4^{+}$, $B0CD8^{+}$ and $BoWCl^{+}$ T cells was conducted using monoclonal antibodies (MAb) against T cell markers to analyze the effect of mastacure, a homeopathic medicine in treatment of mastitis by studying phenotypic distribution of T cells subpopulation in the milk of mastitic buffaloes.

MATERIAL AND METHODS - Five animals suffering from sub clinical mastitis (SCM) were treated with the medicine @ 30 drops orally thrice a day for 20 days. Five normal animals were kept as control and subjected to same treatment with mastacure Untreated group. For laboratory examination, milk samples were collected once before treatment and then on days 10, 20 and 30 after start of therapy. Leucocytes were the population of different cell viz B0CD4⁺ B0CD8⁺ and B0WC1⁺ T cells were analyzed by PACS ^Fluorescent activated cell shorter) using monoclonal antibodies (MAb) against the T cell subpopulation by flowcytometry as per the method of Sharma *ct al.* (1990). A FACS calibur flow cytometer (Becton-Dickin- son) and Hewlett Packard Software wore used for data acquisition, arid analysis of MAb leukocyte staining patterns

RESULTS AND CONCLUSION - The present study demonstrated that after the start of homeopathic treatment there was a gradual decrease in $BoCD8^+$ T cells on day 5 and 10. However, on day 20 there was significant increase in this cell subpopulation. Earlier reports suggested that $CB8^+$ lymphocytes activated during bacterial infection can suppress important host immune responses Holly *et al.*, 1988; Hisatsune *et al.*, 1990; Park *et al.* 1993) and subsequently, the large proportion of $CD8^+$ lymphocytes relative to $CD4^+$ lymphocytes may suppress the activity of these cells', thus contributing to delayed host immune responsiveness during the early stages of pathogenesis. It appeared that the up regulation of BoCD4" T cells on different days following mastacure treatment reported in the present study might be responsible

for enhanced bacterial clearance from mammary gland as revealed by absence of bacteria in milk following treatment with mastacure. There are evidences which showed that, bovine lymphocytes mediate direct antibacterial activity and this activity is dependent on IL-2 activation, an important cytokine, secreted by $CD4^+$ T cells (Shafer- Weaver *et al.* 1996 and Sordillo *et al.*, 1991).

Before treatment

Table 1.		Sequential changes in the proportions of bOCD8 positive T-										
		lymphocytes in the milk samples of normal and mastitic buffaloes										
		before and after administration of mastacure.										
Status		Before	Treatme	ent		Days post treatment						
			5th Day		10th Day		20th day		30th day			
	Mean	t _{cal}	Mean	t _{cal}	Mean	t _{cal}	Mean	t _{cal}	Mean	t _{cal}		
	±SE		±SE		±SE		±SE		±SE			
Normal	13.6	1.56	10.97 ^{ab}	0.36	9.13 ^{ab}	1.16	6.71	3.52*	13.39	1.73		
	<u>+</u> 1.71		<u>+</u> 2.85		<u>+</u> 2.27		<u>+</u> 0.10		<u>+</u> 1.55			
SCM	19.91		12.4		13.89		21.19		18.22			
	<u>+</u> 3.66		<u>+</u> 2.82		<u>+</u> 3.41		<u>+</u> 4.11		<u>+</u> 2.32			

CD at 5% = 6.08

1. * significant (P<0.05)

2. ** highly significant (P < 0.01)

3. Mean with different superscripts differ significantly.

The present study revealed that on day 5 post treatment only two quarters were having

streptococcal infection and showed simultaneous increase in CD4+ cells. On day 10, 20 and post treatment only two, three and one quarter, respectively were found culturally positive for Str agalatiae whereas by one quarter on these days showed isolation of S. aureus. Our study revealed that there was significant up regulation of CD4+ cells from day 5 to day 10 as compared to CD8+. It seems that homeopathic medicine was exerting a synergistic effect in increasing CD4+ cells. Activated CD4+ lymphocytes can then secrete interleukin, such as 1L-.2,

which can convert macrophages into potent effecter cells. (Kalish and Schlossman, 1985). Consequently, these macrophages trigger mitrobial defense mechanism during early phase of disease (Kaufmarin, 1993; Nickerson, 1985) leading to active phase of recovery.

Table 1.		Sequential changes in the proportions of bOCD4 positive T-										
		lymphocytes in the milk samples of normal and mastitic buffaloes										
		before and after administration of mastacure.										
Status		Before	e Treatme	ent		Days post treatment						
			5th Day	10th Day			20th day		30th day			
	Mean	t _{cal}	Mean	t _{cal}	Mean	t _{cal}	Mean	t _{cal}	Mean	t _{cal}		
	±SE		±SE		±SE		±SE		±SE			
Normal	8.85	3.2	11.45	8.79	12.81	4.0	10.92	3.70	11.24	0.3		
	±0.58	7*	±0.62	**.	±0.33	2*	±1.25	*	±2.59	7		
SCM	14.22 ^{bc}		2 5.15ª		26.86s		21.6s		12.67°			
	±1.53		±1.42		±3.48		±2.59		±2.87			

CD at 5% = 7,37 significant (P<0.0S)

- **2.** highly significant (P < 0.01)
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However, further studies are required to elucidate the functional significance of up regulation of BoCD4⁺T cells subpopulation following homeopathic treatment, particularly cytukine profiling following mastacure treatment which will reveal the immunological basis of homeopathic treatment in cure of mastitis

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PROCEEDINGS OF THE 8th WORLD BUFFALO CONGRESS, CASERTA, OCTOBER 19-22, 2007

Guest Editors: Rossella Di Palo (coordinator), Vittorio Baribieri, Giuseppe Campanile, Federico Infascelli, Vincenzo Piccolo



Flow cytometry analysis of different T cell markers in mastitic buffalo milk before and after treatment with Homeopathic medicine

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Key words: Flow cytometry, BoCD4⁺ T Lymphocyte, BoCD8⁺ T Lymphocyte, Mastacure.

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MATERIAL AND METHODS - Five animals suffering from sub clinical mastitis Pleasi (SCM) were treated with the medicine @ 30 drops orally thrice a day for 20 days. Five the days normal animals were kept as control and subjected to same treatment with mastacure as that in treated group. For laboratory examination, milk samples were collected once before treatment and then on day 5, 10, 20 and 30 after start of therapy. Leucocytes were isolated from milk on histopaque gradient. The population of different T cell viz. BoCD4⁺, BoCD8⁺ and BoWC1⁺ T cells were analyzed by FACS (Fluorescent activated cell shorter) using monoclonal antibodies (MAb) against the T cell subpopulation by flowcytometry as per the method of Sharma *et al.* (1990). A FACS calibur flow cytometer (Becton-Dickinson) and Hewlett Packard Software were used for data acquisition, and analysis of MAb leukocyte staining patterns.

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Sequential changes in the proportions of bOCD8 positive T-lymphocytes in Table 1. the milk samples of normal and mastitic buffaloes before and after administration of mastacure.

Status	Before treatment				 Days post treatment 					
And Market Market and A	- <u>1</u> .11		5 th day		10 th day		20 th day		30 th day	
Part of the Part o	Mean ±SE	t _{cal}	Mean ±SE	t _{cal}	Mean ±SE	t _{cal}	Mean ±SE	t _{cal}	Mean ±SE	t _{cai}
and the longer Normal	13.60 ^a ±1.71	1.56	10.97°b ±2.85	0.36	9.13 ^{ab} ±2.27	1.16	6.71 ^b ±0.10	3.52*	13.39ª +1.55	1.7 <mark>3</mark>
T.Y. on A. S. SCM	19.91 ±3.66		12.4 ±2.82	Wilaw e	13.89 ±3.41	e melo	21.19 ±4.11	4.	18.22 ±2.32	
A MARKET BECKENNING						-				

CD at 5% = 6.08CARLEN BOOK STREAM

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VIII WORLD BUFFALO CONGRESS

Sequential changes in the proportions of bOCD4 positive T-lymphocytes in Table 2. the milk samples of normal and mastitic buffaloes before and after administration of mastacure.

Status	Before tr	eatmen	t		Days post treatment								
			5 th day		10 th day		20 th	day	30 th day				
	Mean ±SE	t _{cal}	Mean ±SE	t _{cal}	Mean ±SE	t _{cal}	Mean ±SE	t _{cat}	Mean ±SE	t _{cal}			
Normal	8.85 ±0.58	3.2 7*	11.45 ±0.62	8.79 * *.	12.81 ±0.33	4.0 2*	10.92 ±1.25	3.70 *	11.24 ±2.59	0.3 7			
SCM	14.22 ^{bc} ±1.53		25.15ª ±1.42		26.86ª ±3.48		21.6ª ±2.59		12.67¢ ±2.87				

CD at 5% = 7.37

* significant (P<0.05) ** highly significant (P<0.01)

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However, further studies are required to elucidate the functional significance of up regulation of BoCD4⁺ T cells subpopulation foliowing homeopathic treatment, particularly cytokine profiling following mastacure treatment which will reveal the immunological basis of homeopathic treatment in cure of mastitis.

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Proceedings of the Technical Programme Committee Meeting of the Departments of Central Vety. Laboratory, Teaching Vety. Clinical Service Complex, Animal Biotechnology and Vety. Surgery & Radiology held on 5.8,2005.

The meeting was chaired by Dr. Jlt Singh, Addl. Director of Research and was attended by the Dean and HODs, COVS; HODs of APP, BMB, AN, LPM, Director, TVCSC, Director, NRCE, faculty of all concerned Departments and Associate Director (V&AS), Directorate of Research.

The salient research findings, observations made and decisions taken w.r.t. different research schemes in operation these departments are as under:

Central Vety. Laboratory

Research Schemes:

- 1. C(a)VCL-1-NP(Agri.) " Etiology and diagnosis of mastitis and infectious abortions in animals"
- 2. C(g), COVS-4-OA "Testing of sheep flock for brucellosis"
- 3. C(a)COVS-1-Plan (Agri.) "Establishment of Central Diagnostic Laboratory"

Salient Research Findings 2004-2005:

- Monoclonal antibody based competitive ELISA was standardized and used for detecting brucellosis in cattle.
- Testing of all the 6742 sheep belonging to Central Sheep Breeding Farm for brucellosis was achieved using ELISA. It was more effective in detecting brucellosis infected sheep than RBPT.
- A total 3448 clinical and milk samples (2391 clinical 955 sub clinical mastitis & clinical material) were culturally examined. Resulting isolates were identified and subjected to antimicrobial sensitivity testing.
- Microbial load of 280 semen samples received from different semen banks in Haryana were determined and antibiogram of resulting organism were communicated.
- DAS-ELISA was standardized for detection of staphylococcal mastitis in cows and 279 milk of samples CCSHAU farm were screened using this assay.
- Out of eight diagnostic tests subjected to 100 milk samples, lactate dehydrogenase (LDH) enzyme assay was found to be the best test for detecting sub clinical mastilis in cows, followed by spot TIA (trypsin inhibition assay) and MAMP (modified aulendorfer mastilis probe) test.

Evaluation:

Colors Coste Hall, Elis

The research work under taken during the year 2004-05 was found very go of and evaluated as most satisfactory.

Recommendations generated for field application:

- Spot Trypsin. Inhibition Assay (TIA) and promothymol blue (BTB) plate test can be conducted in the field conditions by the side of a nimals to detect sub clinical mastilis Demonstration and practice of these tests was imparted to fourty field veterinarians
- Mastacure gave encouraging results in treating cases of blood in milk, teat stanosis teal
 obstruction, milk let down problems and sub acute streptococcal mastitis. The drug is
 economical when more than one quarter of animal is affected