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Guest editors:
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+ T lymphocytes 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+ T lymphocytes but 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°D4+, B0CD8+ and BoWC1+ 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 et al. (1990). A FACS calibur flow cytometer (Becton-Dickin- son) and Hewlett Packard Software were 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>
<thead>
<tr>
<th>Status</th>
<th>Before Treatment</th>
<th>Days post treatment</th>
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<tbody>
<tr>
<td></td>
<td>5th Day</td>
<td>10th Day</td>
</tr>
<tr>
<td></td>
<td>Mean ±SE</td>
<td>Mean ±SE</td>
</tr>
<tr>
<td>Normal</td>
<td>13.6 1.56</td>
<td>10.97ab 0.36</td>
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<tr>
<td></td>
<td>+1.71 2.85</td>
<td>+2.27</td>
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<tr>
<td>SCM</td>
<td>19.91</td>
<td>12.4</td>
</tr>
<tr>
<td></td>
<td>+3.66 2.82</td>
<td>+3.41</td>
</tr>
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</table>

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 Stragalatiae 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 IL-2,
which can convert macrophages into potent effector 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.

<table>
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<th>Status</th>
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<tbody>
<tr>
<td></td>
<td>Mean ±SE</td>
<td>5th Day</td>
</tr>
<tr>
<td>Normal</td>
<td>8.85 ±0.58</td>
<td>3.2</td>
</tr>
<tr>
<td>SCM</td>
<td>14.22bc ±1.53</td>
<td>2.5.15s</td>
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CD at 5% = 7.37 significant (P<0.05)

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 cytokine profiling following mastacure treatment which will reveal the immunological basis of homeopathic treatment in cure of mastitis
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Table 2. Sequential changes in the proportions of bOC4D+ T-lymphocytes in the milk samples of normal and mastitic buffaloes before and after administration of mastiure.

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The meeting was chaired by Dr. Jit Singh, Addl. Director of Research and was attended by the Dean and HODs, COVS; HODs of APP, BMB, AN, LPM, Director, TVSCC, 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(Agr.) “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 (Agr.) “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 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 270 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 mastitis in cows, followed by spot TIA (trypsin inhibition assay) and MAMP (modified auclendorf mastitis probe) test.

Evaluation:

The research work undertaken during the year 2004-05 was found very good and evaluated as most satisfactory.

Recommendations generated for field application:

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