In Vivo Effects of a Thymosin a1-Containing Colostral Whey Product on Neutrophils and Lymphocytes from Lactating Cows Without and With Experimentally Induced Staphylococcus aureus Mastitis

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Abstract

Two separate experiments evaluated ID-1 (a commercial bovine whey product containing 5200 pg of thymosin α1/ml) as an immunotherapeutic agent in lactating cows. In the first experiment, cows without mastitis were evaluated for blood leukogram, milk production, total and differential milk cell counts, lymphocyte (Lc) blastogenesis, and neutrophil (PMN) functions (random and directed migration under agarose, chemiluminescence, ingestion of bacteria, iodination, cytochrome C reduction, antibody-independent neutrophil-mediated cytotoxicity, and antibody-dependent cell-mediated cytotoxicity) before and after ID-1 therapy. ID-1 treatment resulted in a significant treatment group by time period interaction for the relative proportion of mononuclear cells (MNC) in milk \( (P<0.009) \) and for PMN random migration \( (P<0.01) \). Based on these interactions, ID-1 treatment appeared to slightly increase the proportion of small MNC in milk and to increase random migration from pretreatment levels by 73% more than increases observed in controls. No significant effect of ID-1 treatment on milk production, total milk somatic cell counts, Lc blastogenesis, or other PMN functions was observed. In cows with experimental \( \text{Staphylococcus aureus} \) intramammary infections, ID-1 treatment resulted in a significant decline in blood leukocyte count \( (P<0.001) \) and blood PMN count \( (P<0.02) \), and maintained PMN random migration \( (P<0.01) \) while controls declined and abrogated a depression in the ability of Lc to respond to mitogens \( (P<0.05) \) that developed in controls as a result of \( \text{S. aureus} \) mastitis. Injection of ID-1 into cows had no adverse effect on their overall health or level of milk production, but did cause subtle and potentially favorable changes in several in vitro immune parameters. In spite of these subtle changes which might indicate increased resistance to mastitis, cows actually developed a more severe \( \text{S. aureus} \) intramammary infection based on a 9% increase in \( \log_{10} \) bacterial shedding in milk.

Disciplines
Veterinary Infectious Diseases | Veterinary Microbiology and Immunobiology | Veterinary Preventive Medicine, Epidemiology, and Public Health

Comments
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In Vivo Effects of a Thymosin \( \alpha_1 \)-Containing Colostral Whey Product on Neutrophils and Lymphocytes from Lactating Cows Without and With Experimentally Induced Staphylococcus aureus Mastitis

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ABSTRACT


Two separate experiments evaluated ID-1 (a commercial bovine whey product containing 5200 pg of thymosin \( \alpha_1 \)/ml) as an immunotherapeutic agent in lactating cows. In the first experiment, cows without mastitis were evaluated for blood leukogram, milk production, total and differential milk cell counts, lymphocyte (Lc) blastogenesis, and neutrophil (PMN) functions (random and directed migration under agarose, chemiluminescence, ingestion of bacteria, iodination, cytochrome C reduction, antibody-independent neutrophil-mediated cytotoxicity, and antibody-dependent cell-mediated cytotoxicity) before and after ID-1 therapy. ID-1 treatment resulted in a significant treatment group by time period interaction for the relative proportion of mononuclear cells (MNC) in milk \( (P<0.009) \) and for PMN random migration \( (P<0.01) \). Based on these interactions, ID-1 treatment appeared to slightly increase the proportion of small MNC in milk and to increase random migration from pretreatment levels by 73\% more than increases observed in controls. No significant effect of ID-1 treatment on milk production, total milk somatic cell counts, Lc blastogenesis, or other PMN functions was observed.

In cows with experimental Staphylococcus aureus intramammary infections, ID-1 treatment resulted in a significant decline in blood leukocyte count \( (P<0.001) \) and blood PMN count \( (P<0.02) \), and maintained PMN random migration \( (P<0.01) \) while controls declined and abrogated a depression in the ability of Lc to respond to mitogens \( (P<0.05) \) that developed in controls as a result of S. aureus mastitis. Injection of ID-1 into cows had no adverse effect on their overall health or level of milk production, but did cause subtle and potentially favorable changes in several in vitro immune parameters. In spite of these subtle changes which might indicate
increased resistance to mastitis, cows actually developed a more severe \textit{S. aureus} intramammary infection based on a 9\% increase in $\log_{10}$ bacterial shedding in milk.

\textbf{INTRODUCTION}

Mastitis is the most costly disease affecting the dairy industry (Bramley and Dodd, 1984). The only proven mastitis control methods are germicidal teat dips and dry cow antibiotic therapy (Bramley and Dodd, 1984). Although prevention is the key to mastitis control, elimination of existing intramammary infections (IMI) by spontaneous cure, treatment, or culling is very important. Treatment of mastitis in lactating cows is fraught with problems: (1) antibiotic residues in milk, (2) reduced efficacy of antibiotic treatment during lactation, and (3) mastitis induced when aseptic technique is not used to administer antibiotics into the mammary gland. Ideally, the best approach to elimination of IMI would be to increase spontaneous cure rates by safely enhancing immune function during periods of peak susceptibility to mastitis. Peak IMI rates occur during the first and last two weeks of a 60-day, nonlactating period (Smith et al., 1985). In addition, clinical mastitis has its highest incidence during early lactation (Malinowski et al., 1983; Syvajarvi et al., 1986).

Thymic hormones have been reported to have restorative effects on immune cell function and to prevent microbial infections in humans and other species (Doria and Frasca, 1984; Ishitsuka et al., 1984; Salvin and Neta, 1984). Thymosin $\alpha_1$, a peptide produced by thymic epithelial cells, is reported to induce maturation of T-helper lymphocytes and enhance neutrophil (PMN) function in mice (Birr, 1984; Bistoni et al., 1985). A product (ID-1) (Immuno-Dynamics, Inc., Perry, IA) derived from bovine colostrum has been developed which has been claimed to reduce the cell count of milk from glands with subclinical IMI and early stages of clinical mastitis. Preliminary data indicated the presence of thymosin $\alpha_1$ in ID-1 (unpublished results, R. Cockrum, Immuno-Dynamics, Inc.). Enhancement of lymphocyte (LC) and PMN function by thymosin $\alpha_1$ may explain the purported efficacy of ID-1 in early or subclinical cases of mastitis.

The purpose of these experiments was to determine the influence of ID-1 administration on peripheral blood LC and PMN function, bacterial shedding and somatic cell counts in milk from mastitis-free, lactating Holstein cows, and cows with experimental \textit{Staphylococcus aureus} mastitis.

\textbf{MATERIALS AND METHODS}

\textit{Preparation of ID-1 from colostrum}

First milking colostrum was obtained from normal, healthy, adult cows and frozen at $-10^\circ\text{C}$ until processed. Thawed colostrum was strained through a
coarse filter, pooled, and heated to 27°C. Rennin and water (up to 20% final volume) were added to remove the casein by enzymatic coagulation. Whey was decanted and passed through a standard dairy high-volume clarifier to remove milk fat. Whey was then passed through a series of filters (two 0.45-μm filters, one 0.22-μm filter), and adjusted to a final pH of 5.4 to 5.6. Protein content was adjusted to 4% (w/v) by dilution with physiologic saline solution. Phenol was used as a preservative at a final concentration of 0.4%.

**Analysis of thymosin α1 content**

Thymosin α1 content of milk, serum, and ID-1 was determined by radioimmunoassay as previously validated for bovine thymosin α1 in bovine tissues (assays performed by Dr. Paul H. Naylor, Dept. Biochemistry, George Washington University Medical Center, Washington, DC, according to procedure of McClure et al., 1982). Milk and serum samples from six Holstein cows were collected from the first, sixth, and twelfth milkings after calving. A prepartum serum sample was also collected. Thymosin α1 content of the ID-1 (Lot # 384) used in this report was also determined.

**Animals and experimental design**

In experiment 1, twelve mid-lactation Holstein cows were randomly assigned into two groups. One group (ID-1) received 40 ml of ID-1 injected subcutaneously daily for 3 consecutive days (total dose of 0.6 μg thymosin α1 per cow); the control group received 3 similar injections of pyrogen-free saline. Peripheral blood Le and PMN functions were evaluated according to the following schedule: Period 1 = Monday, Wednesday, and Friday (MWF) [prior to ID-1 or placebo injections (3 consecutive 10 a.m. injections were given beginning after samples were collected on Friday of Period 1)], Period 2 = Monday through Friday (MTWThF) (5 consecutive days starting 24 h after the final injection), and Period 3 = Monday, Wednesday, and Friday (8, 10, and 12 days after the final injection).

In the week prior to experiment 2, all twelve cows were experimentally infected in one quarter with ~50 colony-forming units of *S. aureus* and divided into two treatment groups with an equal number of cows (3) with or without previous exposure to ID-1 in each group. One group received ID-1, and the second served as the control. Identical treatment and sampling regimens were used in the first and second experiments.

**Hematological studies**

Total leukocyte count in blood was determined by electronic counting of blood collected by jugular venipuncture into tubes containing EDTA. Films of
blood cells were prepared by cytocentrifugation and stained with a combination Giemsa/Wright stain. Approximately 200 cells were differentiated as PMN, eosinophils (PME), or mononuclear cells (MNC). Lymphocytes and monocytes cannot be differentiated in bovine blood by this method, so they were counted together and termed MNC (Hammer and Weber, 1974).

**Milk sample evaluation**

Bacteriological examination of milk samples (~1–2 ml) was done daily from each mammary quarter before the a.m. milking. One-tenth ml of each quarter milk sample was plated onto tryptic soy agar with 5% sheep blood (Micro Bio Logics, St. Cloud, MN) and examined for bacterial growth after 24 and 48 h incubation at 37°C. In the second experiment, milk from known infected mammary quarters was briefly sonicated (~10 s) to break up clusters of staphylococci and to disrupt phagocytes which might contain viable bacteria (Hurley et al., 1985). Serial decimal dilutions of sonicated milk samples were made in 0.015 M phosphate-buffered saline solution (PBSS; pH 7.2), and 0.1 ml of each dilution was plated on duplicate blood agar plates and incubated for 18 h at 37°C. Bacterial colony numbers were expressed as \( \log_{10} \) bacteria/ml of milk.

Total numbers of milk leukocytes in midstream milk were estimated by the direct microscopic method (National Mastitis Council, 1968). Differential cell counts were determined by microscopic examination of cytocentrifuge slide preparations of milk as described above. Daily milk production was recorded throughout the experiments.

**Leukocyte preparation**

Neutrophils and MNC were separated from peripheral blood samples collected into acid citrate dextrose as described (Kehrli et al., 1988a,b). The isolated PMN pellets, which were typically > 95% granulocytes (PMN + PME), were suspended in PBSS at a concentration of 5.0 \( \times \) 10^7/ml for functional analysis. The MNC harvested from density gradient centrifugation (Histopaque 1083, Sigma) were washed twice in PBSS at 400 \( \times \) g for 10 min and then resuspended to a final concentration of 2 \( \times \) 10^6/ml in RPMI 1640 medium containing 25 mM HEPES buffer and 2 mM L-glutamine.

**Neutrophil function assays**

Procedures for evaluating ingestion of \( ^{125} \)I-labeled *S. aureus* (Roth and Kaeberle, 1981a), iodination (Roth and Kaeberle, 1981a), cytochrome C reduction (Canning et al., 1986), antibody-dependent cell-mediated cytotoxicity (ADCC)
(Roth and Kaeberle, 1981b), antibody-independent neutrophil-mediated cytoxicity (AINC) (Roth and Kaeberle, 1981b), native (nonluminol-dependent) chemiluminescence (Kehrli et al., 1988a), directed migration (Lukacs et al., 1985), and random migration under agarose by PMN (Nelson et al., 1975) were done as described with minor modifications (Kehrli et al., 1988a).

Neutrophil adherence was evaluated in the second experiment using $[^{51}\text{Cr}]$-labeled PMN on extracellular matrix-coated, flat-bottomed microtiter plates (Accurate Chemical and Scientific Corp., Westbury, NY). Isolated PMN (1 ml) were incubated for 1 h at 39°C with 7.4 MBq of sodium chromate $[^{51}\text{Cr}]$ (Amersham) in 1 ml Medium 199 (M199) in a humidified atmosphere containing 5% CO₂. After two washings with 10 ml of PBSS, PMN were resuspended to 4 ml with M199 with 25 mM HEPES and 2 mM L-glutamine. Wells of microtiter plates were filled with 100 μl of M199 and prewarmed to 39°C before 100 μl of labeled PMN ($1.25 \times 10^6$) were added. Cells were allowed to adhere for various time periods (0, 15, 30, 45, 60, and 90 min) before non-adherent cells were washed away by three gentle irrigations/aspirations of the well with PBSS (39°C). Fifty microliters of Triton X100 (1% v/v) were added to each well to lyse and release the radiolabel in the remaining adherent PMN. Radioactivity released into the supernatant was then adsorbed by a microtiter supernatant collection system (SCS, Skatron). For analysis, the percent of total $[^{51}\text{Cr}]$-labeled PMN added per well that was adherent was plotted against time, and area under the curve was determined.

**Lymphocyte blastogenesis**

Lymphocyte blastogenesis was performed as previously described (Kehrli et al., 1988b). Media for mitogenic stimulation contained one of the following amounts of mitogen: 25, 12.5 or 6.25 μg of phytohemagglutinin-P (PHAP) (Difco), 15.6, 7.8, or 3.9 μg of concanavalin-A (ConA) (Sigma), or 20, 2, or 0.2 μg of pokeweed mitogen (PWM) (Sigma) per ml of medium. All three concentrations of each mitogen were averaged for an overall mean which was used in statistical analysis.

**Statistical analysis**

Variables were analyzed by fitting the linear model: $y = \text{mean} + \text{group} + \text{cow(group)} + \text{period} + \text{day(period)} + \text{group*period} + \text{group*day(period)} + \text{period*cow(group)} + \text{error}$. In this model, groups were the placebo- and ID-1-treated cows, periods were three individual weeks of each experiment, days were MWF, MTWThF, and MWF in the three periods, and error represented residual animal variation after fitting the above model. The effect of ID-1 treatment is in the group by period ($G*P$) interaction. Therefore, statistical significance was judged by F-tests of the $G*P$ interaction. This model
allows for significant pretreatment differences between groups that may arise by chance, and assumes this difference remains constant over time unless ID-1 treatment imparts a significant effect on the variable analyzed.

RESULTS

*Thymosin α₁ determinations*

Colostrum for the first milking after calving had more than twice as much thymosin α₁ (4100 pg/ml) as did simultaneously collected autologous sera (1900 pg/ml, Fig. 1). By the twelfth milking after calving, the thymosin α₁ content of milk had gradually declined to 2400 pg/ml, while serum content remained the same (2000 pg/ml). The lot of ID-1 used in this report contained 5200 pg of thymosin α₁/ml.

*Experiment 1*

All 12 cows remained free of detectable IMI during the 3 weeks of the first experiment.

Administration of ID-1 to cows without mastitis had no significant G*P effects on blood leukogram values, milk production, somatic cell count, or milk PMN count (data not shown). There was, however, a significant G*P ($P < 0.009$) for the proportion of small MNC in the milk cell population (Fig. 2). This seems due to a slight increase in the relative proportion of small MNC in the milk cell population during the experiment in the ID-1 group. This significant G*P for the proportion of small MNC in milk may be solely due to the large initial difference between treatment groups that arose by chance.

Fig. 1. Thymosin α₁ content of bovine colostrum and serum collected from six Holstein cows at the 1st, 6th, and 12th milkings postpartum. A prepartum serum sample was also evaluated.
Fig. 2. Effect of ID-1 on the mean (±SEM) proportion of small mononuclear cells (presumably lymphocytes) in the milk leukocyte population of two groups of six cows without mastitis. One group was given placebo saline injections and the other was given ID-1 injections on 3 consecutive days before the second period (week) of the experiment.

Neutrophil random migration was found to be significantly affected by ID-1 treatment (G*P; P < 0.001). Administration of ID-1 to healthy lactating dairy cows resulted in a significantly larger proportional increase in PMN random migration during the first week after treatment than the increase of PMN random migration from placebo controls (Fig. 3a). All other PMN functions and Lc blastogenesis results demonstrated parallel values between the two groups for the duration of experiment 1 (data not shown). Therefore, ID-1
administration to cows without mastitis had no significant influence on PMN ingestion of bacteria, PMN oxidative metabolism (measured by chemiluminescence, cytochrome C reduction and iodination), PMN ADCC, or Le blastogenic responses to mitogens (ConA, PHA-P, and PWM).

Experiment 2

There were significant G*P interactions for the effect of ID-1 treatment on the total blood leukocyte count ($P<0.001$) and on the blood PMN count ($P<0.02$) in cows with *S. aureus* mastitis. ID-1 treatment appeared to cause a slight leukopenia from pretreatment values compared to control values (Fig. 4a). The net change in blood neutrophil counts after ID-1 treatment was a slight decline, while the control values went up slightly (Fig. 4b).

Administration of ID-1 to cows with *S. aureus* mastitis did not significantly affect milk production or milk leukocyte values based on the G*P interactions (Table 1). Some interesting trends did appear, however. Milk somatic cell count (SCC) values for the ID-1-treated cows were initially lower than the placebo-injected controls (Table 1). The geometric mean SCC of the control cows had

![Fig. 4. Total blood leukocyte count (a) and blood neutrophil count (b) of two groups of six cows with mastitis. One group was given placebo saline injections and the other was given ID-1 injections on 3 consecutive days before the 2nd period (week) of the experiment. Bars represent group means for each period of the experiment, and lines represent the SE.](image-url)
TABLE 1

Results of various milk parameters measured before and after three daily injections of ID-1 or a placebo into two groups of six cows with experimentally induced mastitis

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Treatment group</th>
<th>Treatment effect</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Controls</td>
<td>ID-1</td>
</tr>
<tr>
<td></td>
<td>Mean ± SEM (n)</td>
<td>Mean ± SEM (n)</td>
</tr>
<tr>
<td>Milk production (kg/d)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>20.6 ± 0.9 (18)</td>
<td>18.7 ± 0.7 (18)</td>
</tr>
<tr>
<td>2</td>
<td>21.0 ± 0.7 (30)</td>
<td>18.8 ± 0.6 (30)</td>
</tr>
<tr>
<td>3</td>
<td>21.3 ± 0.9 (18)</td>
<td>18.6 ± 0.7 (18)</td>
</tr>
<tr>
<td>Milk somatic cell countc (×10^3/ml)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>166 ± 23 (30)</td>
<td>110 ± 10 (30)</td>
</tr>
<tr>
<td>2</td>
<td>302 ± 49 (42)</td>
<td>166 ± 15 (42)</td>
</tr>
<tr>
<td>3</td>
<td>347 ± 48 (42)</td>
<td>224 ± 21 (42)</td>
</tr>
<tr>
<td>Milk neutrophil countc (×10^3/ml)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>55 ± 13 (30)</td>
<td>39 ± 6.3 (30)</td>
</tr>
<tr>
<td>2</td>
<td>160 ± 37 (42)</td>
<td>78 ± 8.9 (42)</td>
</tr>
<tr>
<td>3</td>
<td>150 ± 32 (42)</td>
<td>100 ± 12 (42)</td>
</tr>
<tr>
<td>% Neutrophils in milk cell population</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>45 ± 6 (30)</td>
<td>41 ± 4 (30)</td>
</tr>
<tr>
<td>2</td>
<td>63 ± 4 (42)</td>
<td>52 ± 3 (42)</td>
</tr>
<tr>
<td>3</td>
<td>53 ± 4 (42)</td>
<td>51 ± 4 (42)</td>
</tr>
<tr>
<td>% Small mononuclear cells in the milk population</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>55 ± 6 (30)</td>
<td>59 ± 4 (30)</td>
</tr>
<tr>
<td>2</td>
<td>36 ± 4 (42)</td>
<td>47 ± 3 (42)</td>
</tr>
<tr>
<td>3</td>
<td>46 ± 4 (42)</td>
<td>49 ± 4 (42)</td>
</tr>
<tr>
<td>Bacteria shed in milk (log_{10} cells/ml)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>5.1 ± 0.36 (18)</td>
<td>4.5 ± 0.30 (18)</td>
</tr>
<tr>
<td>2</td>
<td>4.8 ± 0.22 (30)</td>
<td>5.0 ± 0.11 (30)</td>
</tr>
<tr>
<td>3</td>
<td>5.1 ± 0.32 (18)</td>
<td>4.9 ± 0.11 (18)</td>
</tr>
</tbody>
</table>

*Period 1 = Mon., Wed. and Fri. before treatment; Period 2 = Mon. through Fri. after the last treatment; Period 3 = Mon., Wed. and Fri. after period 2.

bNS = not significant (P > 0.05).

cGeometric mean cell counts and SEM.

increased 182% by the week after placebo injections as the S. aureus infections progressed. This increase represented a 31% larger proportional increase in the SCC than what was observed in the ID-1-treated group. This difference was not significant, however, as judged by the G*P. The relative proportion of PMN in the SCC and absolute number of PMN/ml of milk increased to a larger value in the control cows than in the ID-1 group (Table 1). This was not significant, however. The proportion of small MNC in the milk cell population appeared to decline to a lesser degree in the ID-1-treated cows. Although this was not significant, it does agree with the results of our first experiment where
a slightly higher value was found for the proportion of small MNC in the milk cell population of ID-1-treated cows without IMI.

Every quarter with an experimentally-induced IMI remained infected, based on daily culture results. The range of bacterial shedding values (bacteria/ml milk) was consistent for a given cow. The $\log_{10}$ bacteria/ml range for all quarters of all cows was 0 to 7. There was a significant $G \times P$ ($P < 0.008$) for bacterial shedding into milk from infected quarters (Table 1). During the initial period after ID-1 treatment, there was a 9% $\log_{10}$ increase in bacterial shedding from the udder into the milk of cows receiving ID-1, while the controls experienced a 7% $\log_{10}$ drop in shedding of $S. aureus$.

In experiment 2, a significant $G \times P$ was observed for the effect of ID-1 treatment on PMN random migration ($P < 0.01$). ID-1 given to lactating dairy cows with mastitis the week after treatment resulted in higher PMN random migration values than control values (Fig. 5). No other effects of ID-1 treatment on PMN function were detected (data not shown).

ID-1 treatment had a significant $G \times P$ ($P < 0.05$) effect on Lc blastogenesis stimulated by ConA and PHAP (Fig. 6). The effect of ID-1 treatment was to abrogate a depression in the ability of Lc to respond to mitogens that developed in the controls as $S. aureus$ mastitis progressed (Fig. 6). This effect is less convincing for ConA due to the large pretreatment value difference between the two groups (Fig. 6b).

![Fig. 5. Random migration under agarose activity of blood neutrophils collected from two groups of six cows with experimentally induced Staphylococcus aureus mastitis. One group was given placebo saline injections and the other was given ID-1 injections on 3 consecutive days before the 2nd period (week) of the experiment. Points represent group means (±SEM) for each period of the experiment.](image-url)
Fig. 6. Uptake of [³H]-thymidine by phytohemagglutinin- (a) and concanavalin A- (b) stimulated cultures of blood mononuclear cells collected from two groups of six cows with experimentally induced *Staphylococcus aureus* mastitis. One group was given placebo saline injections and the other was given ID-1 injections on 3 consecutive days before the 2nd period (week) of the experiment. Points represent group means (±SEM) for each period of the experiment.

**DISCUSSION**

Thymosin α₁ was found to be present in bovine colostrum at levels more than twice as high as levels found in autologous sera. In addition, the thymosin α₁ content of the commercial whey product used in this study was slightly more than for colostrum. Bovine thymosin α₁ is an acidic peptide (M.W. 3108) with a pI of 4.2 (Goldstein et al., 1977). In humans, the concentration of thymosin α₁ in blood is highest in utero and decreases sharply after birth (McClure et al., 1982). Maternal blood levels of thymosin α₁ also appear to be above normal during gestation in humans (McClure et al., 1982). High fetal blood levels of thymosin α₁ may contribute to these elevated maternal serum levels. The importance of thymosin α₁ in bovine colostrum to the suckling calf is not known. The acidic nature of bovine lacteal secretions (pH 6.6–7.2) compared to blood (pH 7.35–7.5) (Duncan and Prasse, 1977) may result in ion-trapping of thymosin α₁ in colostrum. An exhaustive compositional analysis of ID-1 has not been done to identify all of its biologically active components. Thymosin α₁ is just one of many possible, biologically active molecules in colostrum other than antibodies.
Biologic activity was detected for ID-1 in cows with *S. aureus* mastitis; however, the biologic significance of the observed changes on the outcome of mastitis is not known. ID-1 therapy did not significantly reduce the somatic cell count in milk from *S. aureus*-infected glands and was not effective as a sole therapeutic agent for experimentally induced *S. aureus* mastitis.

Administration of ID-1 to apparently healthy cows resulted in a significant increase in PMN random migration. This effect can be interpreted as a loss of PMN stickiness and may reduce PMN egress from the blood stream into tissues (Clark et al., 1979; Harmsen and Turney, 1985). In normal healthy animals, it is not known if this change may affect susceptibility to infectious disease such as mastitis. There were no other significant effects of ID-1 on peripheral blood PMN or Lc function detected in healthy cows. Most immunotherapeutic agents are intended to restore a faltering immune system. The primary objective of this form of therapy is to optimize host cellular immune responses. The lack of significant effects on immune cell function in healthy animals does not rule out the possibility of an immunotherapeutic agent enhancing immune function in an animal with comprised immune function.

Cows in the ID-1 treatment group demonstrated a slight increase in the proportion of small MNC in the milk cell population (Fig. 2). Lymphocytes produce lymphokines which can enhance phagocyte (PMN and macrophage) function in the bovine (Lukacs et al., 1985; Steinbeck et al., 1986). Whether this slight increase has any functional significance in the mammary gland remains to be determined.

ID-1 administration to cows infected with *S. aureus* in one or more quarters maintained PMN random migration, while the control's values declined (Fig. 5). This is closely related to the observed increase in PMN random migration in cows without mastitis (Fig. 3). This activity is similar to the effect of glucocorticoids in cattle (an increase in PMN random migration) (Roth et al., 1982). However, the typical neutrophilia and depressed PMN iodination and Lc blastogenesis in cattle responding to glucocorticoids was not observed. We also observed a trend in the milk somatic cell count that might indicate fewer total leukocytes entering the mammary glands of the ID-1-treated infected cows as compared to the infected control cows (Table 1). The PMN adherence assay used in the second experiment did not indicate any loss of PMN adherence. Our adherence assay performed on extracellular matrix-coated plates had a higher percentage of adherent PMN (32-40% adhering) than a previous assay using plain polystyrene plates (24% adhering) (Lukacs et al., 1985).

As shown in Fig. 6, in infected control cows there was suppression of Lc blastogenesis which is in agreement with a previous report on cows with chronic *S. aureus* mastitis (Nonnecke and Harp, 1985). Impairment in bovine peripheral blood Lc function has also been associated with other types of mastitis (Kashiwazaki, 1984). Suppression of Lc blastogenesis was not as marked in
the ID-1-treated cows \((P < 0.05)\), indicating a potential immunotherapeutic effect of ID-1 on Lc (Fig. 6). This effect for ConA may be in part due to initial chance differences between the two groups of cows. However, the effect of ID-1 on PHAP-induced Lc blastogenesis supports the idea of a significant, short-term enhancement of Lc function. The role of milk Lc is not clearly defined, but it is plausible that they may affect PMN function for elaboration of lymphokines (Harmsen and Turney, 1985; Lukacs et al., 1985).

Increased bacterial shedding from the udders of cows treated with ID-1 coincided with the time when Lc blastogenesis was sustained by ID-1 therapy. The biological significance of this increase in bacterial shedding is unknown. One can infer from this finding that ID-1 treatment actually worsened the IMI. This interpretation is a paradox to the more favorable effects on Lc blastogenesis. Our bacterial shedding values include bacteria free in the milk and remaining viable bacteria released by sonic disruption of phagocytes in milk. Increased bacterial shedding could represent higher bacterial numbers in milk because of fewer phagocytes going into the gland to control bacteria, or more bacteria being phagocytosed and perhaps cleared from the udder by milk PMN and macrophages. The mild sonication of milk samples prior to bacterial enumeration was a very effective technique which yielded uniform and reproducible results from individual cows over the duration of the experiment. Nine of the 13 quarters evaluated for 19 consecutive days never deviated more than one log$_{10}$ value from the mean shedding values. In addition, only one of 247 total quarter samples cultured came up with no \textit{S. aureus} isolation. We believe sonication of milk samples is a valuable technique to improve the sensitivity of bacterial culturing. Milk somatic cell counts were lower than we had anticipated for cows with \textit{S. aureus} mastitis (e.g. > 600 000 cells/ml), which may have reduced our ability to detect any major effect ID-1 might have on cows with higher somatic cell counts.

Immunotherapeutic agents often have subtle effects which sustain immune cell functions that may be faltering; effects of ID-1 on Lc and PMN function fit this description although all observed changes were small. A close relationship between altered immunity and disease occurrence exists for mastitis and other diseases of cattle (Jain et al., 1968; Guidry et al., 1976; Newbould, 1976; Schalm et al., 1976; Hill et al., 1979; Hoerlein, 1980; Hill, 1981; Lofstedt et al., 1983; Gunnink, 1984; Kashiwazaki, 1984; Kehrli et al., 1988a,b). Enhancement or restoration of the immune system with immunotherapeutic agents could increase resistance to mastitis. It is possible that we did not adequately immunosuppress the cows in our experiments and therefore failed to detect more restorative effects of ID-1 on the bovine immune system. Periparturient cows (Kashiwazaki, 1984; Kehrli et al., 1988a,b) are more immunosuppressed than the cows with \textit{S. aureus} mastitis in this report and might offer the opportunity to see greater effects of potential immunomodulator treatment.
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REFERENCES


