Duralactin®

technical monograph

Only Duralactin® products contain MicroLactin®, a protein concentrate from the milk of hyperimmunized cows.

PRN® PHARMACAL

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Introduction

Inflammation: A Double-Edged Sword

Acute inflammation protects the body from invading microorganisms and injury. It is a rapid-onset, non-specific defense that responds in a similar fashion to all types of foreign organisms and injuries.

In most cases, acute inflammation resolves quickly after eliminating the inciting cause. However, if inflammation does not subside normally, it can result in chronic inflammation. Chronic inflammation has no beneficial purpose and damages healthy tissues. It typically progresses slowly and can result in permanent tissue damage. For example, osteoarthritis can cause cartilage loss and osteophyte formation, and hepatitis can lead to cirrhosis. These permanent changes cannot be reversed. Inflammation also causes pain and impairs a patient's quality of life. Additionally, localized inflammation can have systemic effects that can affect other organ systems and overall health.

Veterinarians use powerful anti-inflammatory drugs—such as corticosteroids and nonsteroidal anti-inflammatory drugs (NSAIDs)—to combat the destructive effects of chronic inflammation. However, the risk of side effects associated with these drugs limits their use in some patients and leads veterinarians to look for alternatives in form of nutraceuticals and supplements.

Duralactin® brand products are nutritional supplements for the long-term management of inflammation in cats, dogs, and horses. The active ingredient in Duralactin® products is MicroLactin®, which is a protein concentrate from the milk of hyperimmunized cows. It contains hyperimmune milk factor (HIMF), a specialized factor that has been shown to reduce inflammation by inhibiting neutrophil migration. This technical monograph will review the inflammatory process, neutrophil migration, MicroLactin®, hyperimmunization, the published target animal studies in dogs and horses, and the mechanism of action of HIMF.

Duralactin® (MicroLactin®) Technical Monograph

Contents

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The Inflammatory Process[1,2]

Immediately after an injury occurs, local mast cells, dendritic cells, and resident macrophages alert the immune system to the presence of damaged cells or foreign organisms. These sentinel cells release cytokines (such as interleukin 1 and interferon α), kinins (such as bradykinin), and vasoactive amines (such as histamine) to trigger the inflammatory process. These and other inflammatory mediators are the chemical communication signals that direct the entire inflammatory process.

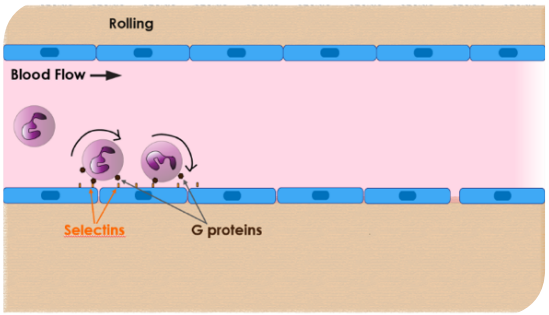
Acute inflammation ensues with two phases of inflammation: vascular and cellular. The vascular stage of inflammation consists of vasodilation and increased vascular permeability. The cellular stage of inflammation involves the migration of leukocytes, predominantly neutrophils, into the affected tissues.

After entering inflamed tissues, neutrophils eliminate foreign organisms via phagocytosis. They also release toxic substances, such as degradative enzymes (elastase and collagenase), reactive oxygen species, and proinflammatory mediators. The proinflammatory mediators released by neutrophils amplify the inflammatory process by stimulating the bone marrow to produce more neutrophils, recruiting circulating neutrophils to the site of inflammation, and helping stimulate the arachidonic acid metabolism cascade. Circulating macrophages then migrate into the tissues to eliminate damaged cells and dying neutrophils and to help heal tissues.

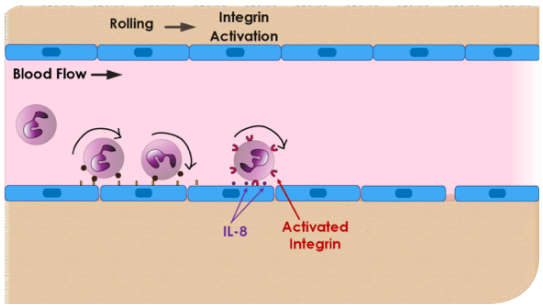
Neutrophil migration[3]

Because of the toxic effects caused by neutrophils once they enter the tissues, it is imperative that the body tightly regulate their ability to do so. Neutrophil migration requires a series of sequential steps: rolling, integrin activation, firm attachment, transmigration, and chemotaxis.

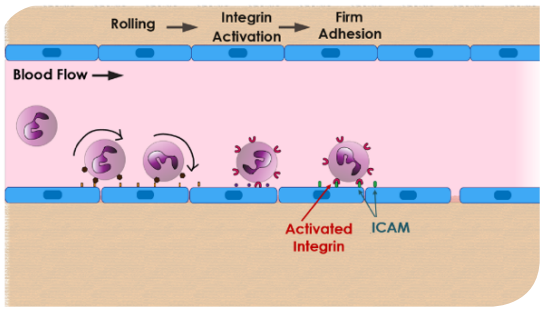
Rolling: Circulating neutrophils come in contact with the blood vessel wall via loose interactions between selectin receptors on endothelial cells and glycoproteins on the neutrophil. This is a weak interaction, and the force of the blood flow is able to break the bond just as another forms a little farther down the endothelium. As the neutrophils forms, breaks, and then forms new bonds with the selectin receptors, it appears to roll along the endothelial surface.



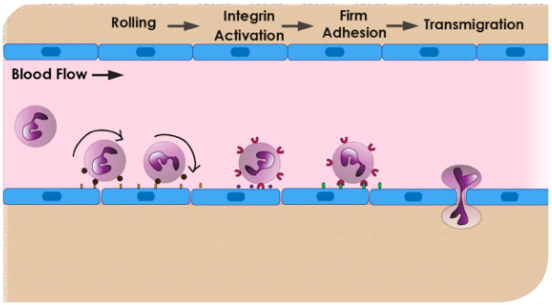
Integrin activation: As neutrophils roll along the endothelium, they come into contact with the proinflammatory chemokine called IL-8, which is expressed by endothelial cells during times of inflammation. IL-8 binds to chemokine receptors on neutrophils, which triggers an intercellular cascade within the neutrophil. This causes the integrin receptor on the surface of the neutrophil to adopt an active conformation.



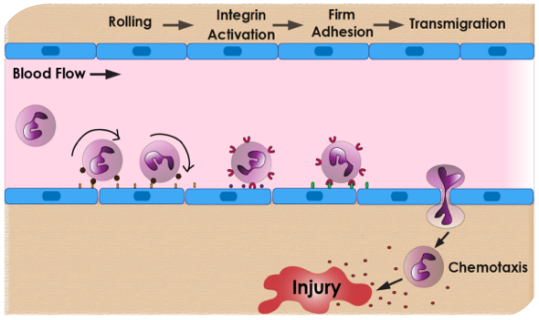
Firm adhesion: The activated integrin receptors on neutrophils bind strongly to intercellular adhesion molecules (ICAM) on the endothelial surface. This interaction is stronger than the force of blood flow, and the neutrophil stops rolling and adheres to the endothelial wall.



Transmigration: Once firmly adhered, the neutrophil crawls to the closest endothelial cell border. The cytoskeleton within the neutrophil reorganizes, changing its shape dramatically. A leading edge of the neutrophil then inserts itself between two endothelial cells, allowing it to exit the circulation and enter the underlying connective tissue. This process is referred to as transmigration, diapedesis, or transendothelial migration.



Chemotaxis: Once the neutrophil enters the affected tissue, it follows a gradient of chemokines, microbial products, and molecules from damaged cells to the site of inflammation.



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Milk: Defending Infants from Inflammation

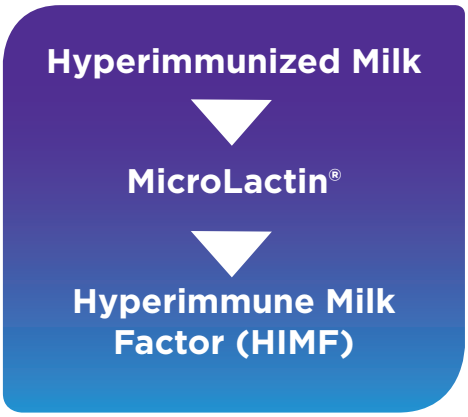
In addition to providing infants with complete and balanced nutrition, a mother's milk contains a number of protective properties. Maternal immunoglobulins defend the young against specific microorganisms. Other components of milk, such as lactoperoxidase and lactoferrin, provide non-specific protection against a range of microorganisms. Antioxidants found in milk decrease tissue damage caused by free radicals.[4] Compared to formula-fed human babies, studies have shown that breastfed babies have a decreased risk for digestive illnesses, ear infections, respiratory infections, asthma, allergies, obesity, and childhood leukemia.[5] Importantly, the maternal immunity delivered through milk is necessary for infant survival in many species.[4]

Hyperimmunized milk[4,6]

In 1976, researchers discovered that dairy milk also contains a natural anti-inflammatory substance during times of infection. This factor is secreted in milk in order to protect the calf from the tissue damage caused by inflammation triggered by bacterial and viral infections. However, the factor is not present normally in milk, only during times of infection. The researchers induced healthy cows to produce this factor by frequently vaccinating them with killed, polyvalent bacterial vaccines, a process called *hyperimmunization*. Hyperimmunized milk contains an orally active factor—most frequently called *hyperimmune milk factor (HIMF)*—that has been shown to reduce inflammation in a number of species. A review of these studies can be found on pages 12-24 of this monograph.

MicroLactin®

MicroLactin® is a protein concentrate of hyperimmunized milk in which the lactose and salts have been reduced. A variety of assays have shown that it retains the biologically active properties of hyperimmunized milk.[7] MicroLactin is the active ingredient of Duralactin® brand products. The published papers that have examined the use of MicroLactin® in managing inflammation in dogs and horses are reviewed in the next section.



MicroLactin is generally well tolerated in dogs, cats, and horses. Because it is derived from milk, side effects can occur in patients with dairy intolerances. Side effects tend to be limited to gastrointestinal upset, such as vomiting (companion animals) and diarrhea.

Target Animal Studies: Canine Efficacy Study[8]

Gingerich DA, Strobel JD. Use of Client-Specific Outcome Measures to Assess Treatment Effects in Geriatric, Arthritic Dogs: Controlled Clinical Evaluation of a Nutraceutical. *Veterinary Therapeutics.* 2003; 4(1): 56-66.

Summary

The efficacy of special milk protein concentrate (SMPC, also known as MicroLactin®) was evaluated in a placebo-controlled, double-blinded, randomized, parallel trial of 50 geriatric, large breed dogs. Overall improvement was seen by owners in 67% of the MicroLactin-treated dogs, whereas only 35% of the owners of dogs in the placebo group reported improvement during the 8-week study period. The treatment group also displayed a greater degree of improvement than the placebo group in regard to orthopedic score ($P < 0.001$) and owner global assessments ($P = 0.004$).

Objective: Evaluate the efficacy of special milk protein concentrate (SMPC, also known as MicroLactin®) in geriatric, large-breed dogs with signs of osteoarthritis.

Study design

Test population: Fifty, client-owned, large-breed dogs ranging from 7 to 12 years of age were randomly assigned to either the treatment or control groups. All participants displayed clinical signs consistent with osteoarthritis. Obese patients or those with underlying disorders that required overlapping treatments (e.g., NSAIDs, steroids, analgesics) were excluded. Five companion-animal practices in the Cincinnati area evaluated the dogs on an outpatient basis.

Treatments: The treatment group received 2 grams of SMBI (MicroLactin®) per day, whereas the placebo group received rice flour. The study period lasted 8 weeks after a 1-week placebo run-in period. The capsules and bottles were identical, and veterinarians and owners were blinded to the group assignments.

Evaluations: Owners evaluated their dogs utilizing an overall assessment, a standardized questionnaire, and a case-specific questionnaire. Veterinarians assigned a global assessment score and performed physical exams at 0, 4, and 8 weeks. A complete blood count and standard chemistry profile were performed at the beginning and end of the treatment period. The global assessment score evaluated whether the patient had improved, which ranged from -1 (worse) to 3+ (excellent). The standardized questionnaire form was identical for all patients. The case-specific questionnaire, which is known as "Cincinnati Orthopedic Disability Index" (CODI), identifies the arthritic impairments specific to each patient's clinical signs. Owners completed both questionnaire types biweekly.

Results

Thirty-five dogs (17 in the placebo and 18 in the treatment group) completed the study. Owners reported an overall improvement in 66.7% of the MicroLactin-treated dogs but in only 35.3% of the placebo-treated dogs. There was also a significant degree of improvement in the MicroLactin-treated group. The owner global assessments and questionnaire scores (both standardized and case-specific) improved significantly in the MicroLactin® group over the course of the study ($P < 0.01$). The placebo group did not improve significantly over the course of the study. The owner's overall assessment and the case-specific questionnaire (CODI) scores improved significantly from the placebo group ($P < 0.05$). The physical examination findings of both the treatment and placebo groups improved slightly but significantly. According to published orthopedic standards, MicroLactin® had a large effect on the owner overall assessments and on the case-specific questionnaire scores, intermediate effect on veterinarians' overall assessments and standardized test scores, and negligible effect on the physical examination findings. Both the MicroLactin® and the placebo were well-tolerated, except that one participant from each group needed to withdraw from the study due to vomiting.

Change in Questionnaire Scores

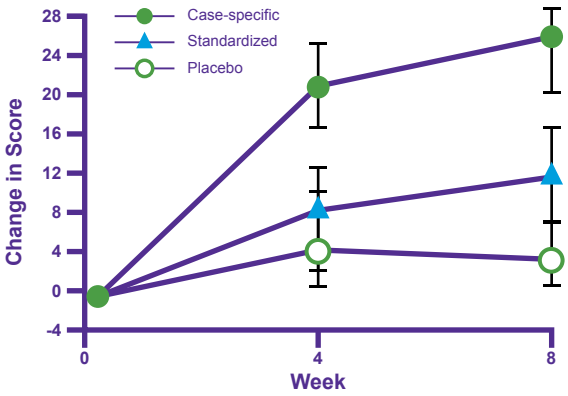


Figure 1: Owner-reported changes (mean +/- SEM) in disability scores of MicroLactin®-treated dogs on the case-specific and standardized questionnaires as compared to the placebo group. Graph borrowed from Gingerich DA, Strobel JD. Use of client-specific outcome measures to assess treatment effects in geriatric, arthritic dogs: controlled clinical evaluation of a nutraceutical. *Veterinary Therapeutics*. 2003; 4(1): 56-66.

Overall Global Response as Seen by Dog Owners and by Veterinarians

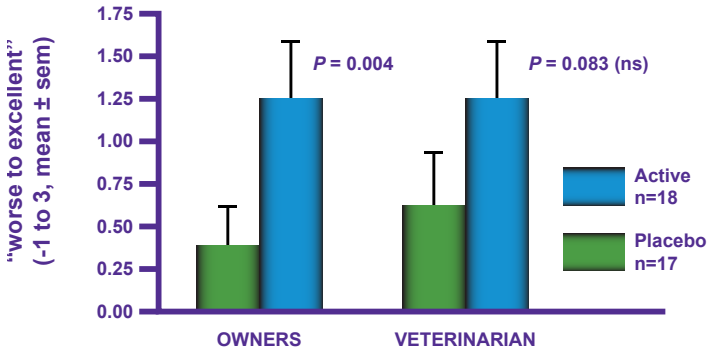


Figure 2: Global assessments of overall response (mean +/- SEM) to MicroLactin® compared to the placebo as reported by owners and veterinarians after 8 weeks. The arthritic dogs were scored on a scale from -1 (worse) to 3 (excellent). Graph adapted from Gingerich DA, Strobel JD. Use of client-specific outcome measures to assess treatment effects in geriatric, arthritic dogs: controlled clinical evaluation of a nutraceutical. *Veterinary Therapeutics*. 2003; 4(1): 56-66.

Significance

This placebo-controlled, double-blinded, prospective, randomized study evaluated the efficacy of MicroLactin® by examining the owner's assessment of their dog's clinical improvement. The results suggest that MicroLactin has therapeutic value in dogs with signs of osteoarthritis. It is a well-tolerated alternative for the long-term management of musculoskeletal conditions, especially in geriatric dogs.

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MicroLactin® and Equine Inflammatory Conditions[9]

Bello TR, Allen TA. The Use of MicroLactin for Inflammatory Conditions in Equine Veterinary Practice. *Journal of Equine Veterinary Science.* 2005; 25(9): 380-382.

Summary

A clinical survey was performed of 58 horses that were treated with Duralactin® Equine for a variety of inflammatory conditions. Additional therapies were included as necessary. Improvement was seen in 86% of the cases.

Objective: Utilize MicroLactin® as a therapeutic for a variety of inflammatory conditions in horses. Conditions that show improvement can be further studied in future placebo-controlled studies.

Materials and Methods

Each horse received 1 scoop of Duralactin Equine (7,000 mg MicroLactin) twice daily with food. Additional therapies were prescribed as necessary. The survey includes a large range of breeds and ages (0.75-36 years). There were 20 mares, 36 geldings, and 2 stallions.

Results and Discussion

Inflammation was reduced in 86% of the cases (44/51). Seven horses were lost to follow-up. The inflammatory conditions included in the survey are listed in Table 1.

Table 1

Inflammatory Conditions in Equine Survey	
AFFECTED SITE	**CONDITION**
Respiratory tract	Small airway inflammatory syndrome
	Allergic rhinitis
Distal limb	Navicular syndrome
	Laminitis
	Hoof reconstruction
	Fetlock pain with soft tissue trauma
	Hock inflammation with joint trauma
Neurologic system	Equine protozoal myeloencephalitis (EPM)
Subcutaneous tissue	Nonpoisonous snake bite
	Multiple tick bites
	Perivulvar inflammation
	Necrotizing vasculitis
Muscle	Traumatic myositis
	Unbalanced shoes
	Hindquarter pressure in training
Skin	Culicoides-bite hypersensitivity
	Topical groin irritation
	Dermatophilus skin infection
	Fibrous tracts and SQ granulomas
Intestines	Toxic enteritis
	Ingestion of toxic plants
Kidney	Undiagnosed
Head	Lacerations

Significance

This clinical survey shows clinical improvement after the supplementation of MicroLactin® in a variety of equine inflammatory conditions. The results can direct future placebo-controlled trials in these and other inflammatory conditions. MicroLactin can affect inflammation throughout the body.

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Equine Protozoal Myeloencephalitis Study[10]

Bello TR, Allen TM. An Intensive Approach in the Treatment of Clinical Equine Protozoal Myeloencephalitis. *Journal of Equine Veterinary Science.* 2008; 28(8): 479-483.

Summary

Twenty-eight horses affected with neurologic signs due to equine protozoal encephalitis (EPM) were treated with a triple therapy of ponazuril, transfer factor, and MicroLactin®. Ponazuril provided antiparasitic therapy, transfer factor stimulated cell-mediated immunity, and MicroLactin inhibited inflammatory reactions. After treatment, 82% (23/28) of the horses were able to return to work.

Many experts consider EPM as "a serious parasitic disease with neurologic consequences" instead of a "serious neurologic disease caused by a parasite." The study's authors wanted to address the host-parasite immunologic reactions associated with the disease by including ponazuril, transfer factor, and MicroLactin in the treatment protocol. Ponazuril is an antiparasitic. Transfer factor contains dialyzable leukocyte extracts that can transfer and stimulate cell-mediated immunity, which is important in controlling intracellular parasites. MicroLactin protects against destructive inflammatory reactions.

Objective: Utilize a triple therapy of ponazuril, transfer factor, and MicroLactin to combat the parasitic, immunologic, and inflammatory components of equine protozoal myeloencephalitis (EPM) in order rehabilitate equine athletes.

Materials and Methods

Twenty-eight horses with clinical signs of EPM—such as gait abnormalities (stumbling), behavior changes, weakness, and asymmetrical muscle loss—exhibited positive serum immunoblot tests to confirm exposure to *Sarcocystis neurona*. A presumptive diagnosis of EPM was made based on clinical signs, a positive serum *immuoblo*t test, and physical examinations; a confirmatory cerebral spinal fluid analysis was not performed. Nine breeds were represented in the study with ages ranging from 3-20 years. There were 15 geldings and 13 mares.

Transfer factor (750 mg twice daily for 7 days, then once daily for 30 days) was fed for a total of 37 days, while both MicroLactin® (7,000 mg twice daily) and ponazuril (5 mg/kg once daily) were given for 28 days. Five horses were treated with ponazuril and transfer factor, and the remaining 23 were treated with a triple therapy of ponazuril, transfer factor, and MicroLactin. Fifteen horses required an extended course of medication.

Results

After treatment, 82% (23/28) of the horses were able to return to work. Eighteen of these returned to their previous activity or were sold as athletes, while five of these horses remained in physical rehabilitation but were able to do controlled exercise under saddle.

Five acutely affected horses were not helped by the therapy. Two of these horses were deemed unsafe and were euthanized within 60 days. The final three made improvements but remained unsafe to ride and were euthanized within 6-24 months of therapy. A treatment crisis occurred in one horse that received only transfer factor and ponazuril.

Significance

Treatment of EPM with ponazuril alone has an expected improvement rate of 60%, with only 10-20% of horses making a complete recovery.[11] With the addition of transfer factor to promote cell-mediated immunity and MicroLactin® to inhibit exuberant inflammation, 82% of horses improved, with 64% making a full recovery. The study's authors "considered transfer factor, MicroLactin, and ponazuril to be equal partners attacking the clinical challenge from different, but specific, directions." Although this study was not placebo-controlled, it suggests an improved treatment response for EPM with the described triple therapy.

The Science of Hyperimmunized Milk: The Mechanism of Action of HIMF

Hyperimmune milk factor (HIMF) is a partially purified factor in hyperimmune milk that has been fractionated from the milk utilizing ultrafiltration techniques. The following studies show that it is HIMF that is responsible for bioactive properties of hyperimmune milk. These experiments examine how isolated HIMF affects inflammation.

Carrageenan-Induced Inflammation

The rat paw edema assay is a classic laboratory model to determine whether a substance can reduce inflammation *in vivo*. In this test, researchers inject carrageenan into one of the paws of laboratory rats. The injection triggers an inflammatory reaction that causes edema, hyperemia, and pain. The amount of swelling is then measured to quantify the amount of inflammation that has occurred. This measurement is then compared to the opposite paw that did not receive a carrageenan injection. An anti-inflammatory effect is demonstrated if there is no significant difference between fluid volumes of the test paw and that of the untreated paw.

Rat paw edema assays typically also include a positive control group. A known anti-inflammatory agent (such as aspirin) confirms the inflammatory action of the carrageenan. The effects of the tested substance can then be compared to the effects of the negative control (no treatment, water, or saline) and the positive control groups.

Numerous rat paw edema assays have shown that HIMF reduces inflammation.[4,6,12,13,14] In these experiments, HIMF was administered intravenously (IV), intraperitoneally (IP), intramuscularly (IM), subcutaneously (SQ), or orally (PO) to treatment rats. Other control rats were administered either aspirin, water, or normal milk. The paws of rats treated with HIMF showed significantly less swelling than the paws of the rats that received normal milk or water (see Figure 3). Also, the carrageenan-injected paws of rats treated with HIMF were statistically no different from the paws that were not injected (see Figure 3), confirming an inhibition of the inflammatory response. Similar findings have been confirmed repeatedly with many follow-up experiments and show that HIMF can be used to reduce inflammation in animals. The results also confirm that HIMF can reduce inflammation when given orally (see Figure 4).

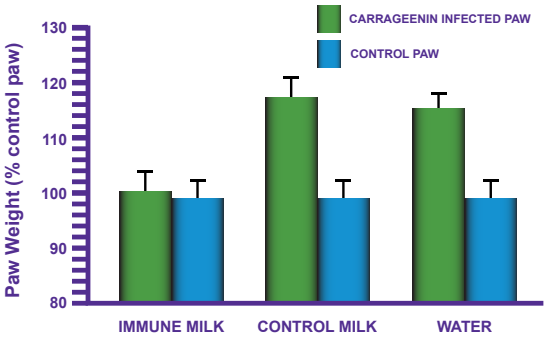

Figure 3. Effect of IP hyperimmune milk factor (HIMF) on tissue swelling caused by carrageenan in the rat paw (mean +/- sem, n=10). The control paw did not receive any carrageenan. Graph borrowed from Beck LR, Fuhrer JP. Anti-inflammatory factor, method of isolation, and use. US Patent #5980953. Nov1999:Fig.3.

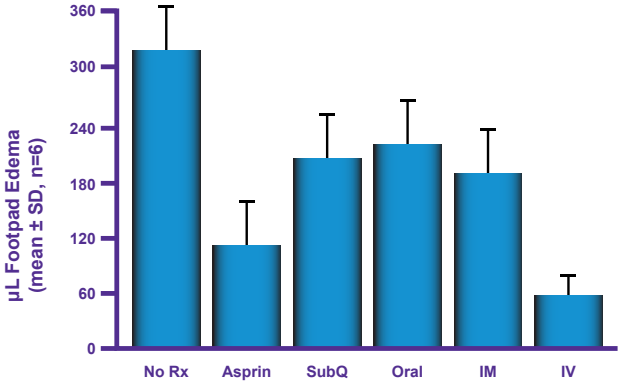

Figure 4. Effect of subcutaneous (SubQ), oral, intramuscular (IM), and intravenous (IV) hyperimmune milk factor (HIMF); control (no Rx); and aspirin on tissue swelling caused by carrageenan in the rat paw. Graph borrowed from Woods C, Gingerich D. Technical Brief: Pharmacology of MicroLactin®.

The swelling in response to the carrageenan occurs in two distinct stages. The first phase of swelling occurs because of the actions of several inflammatory mediators (eg, bradykinin and histamine); the second phase occurs because of the actions of inflammatory cells, especially neutrophils. An additional experiment showed significant effects of HIMF during the second, but not the first, stage (see Figure 5). This indicates that HIMF appears to decrease the effect of inflammatory cells: neutrophils and macrophages.[13]

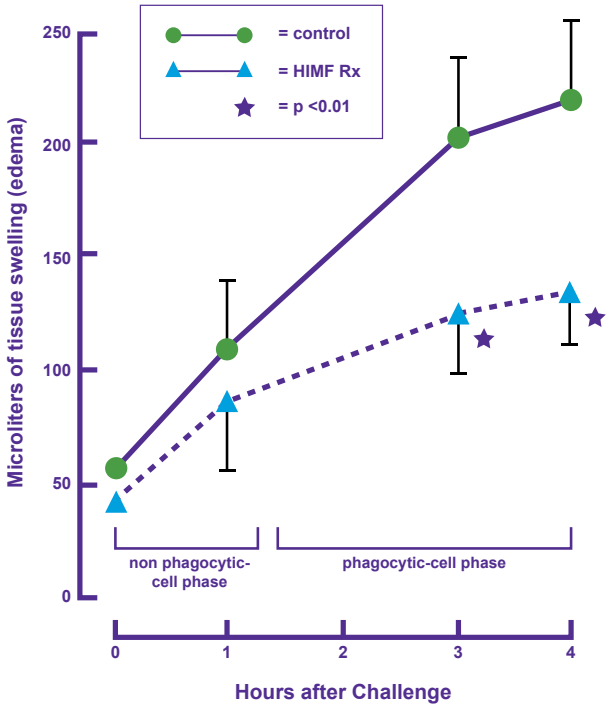

Figure 5. Volume (in µL) of tissue swelling in response to carrageenan injections during a rat paw edema test. The control group received saline, and the hyperimmune milk factor (HIMF) group received 40 mg of intravenous HIMF. The Wilcoxon sum of ranks test was used to determine a significant difference (* = $P < 0.01$) between the 2 groups during the stage in which inflammatory cells act. Graph is adapted from Ormrod DJ, Miller TE. A low molecular weight component derived from the milk of hyperimmunized cows suppresses inflammation by inhibiting neutrophil emigration. *Agents and Actions*. 1992;37:73. Figure1.

The pleural neutrophil migration inhibition assay also utilized carrageenan to test the action of HIMF. In this test, scientists injected carrageenan into the pleural cavity of rats causing neutrophils to rush to the area. They then immediately injected 3 mg HIMF IP, which inhibited neutrophil migration by more than 70% after four hours. The control rats showed only 18% inhibition. Therefore, HIMF appears to inhibit the number of neutrophils present at an inflammatory site.[6]

Reverse Passive Arthus Reaction

In the reverse passive Arthus reaction (RPA), rats receive IV injections of ovalbumin (antigen) and intradermal antiserum to ovalbumin (antibodies) derived in rabbits. At the site of the intradermal injections, the antibodies bind to the antigens and create an intense inflammatory reaction. Because neutrophils are primarily responsible for the reaction, medications that inhibit inflammation in a RPA test are thought to affect the function of neutrophils.[6]

In an RPA experiment, rats treated with 20 mg of IV HIMF showed 81% fewer neutrophils, 44% less tissue swelling, and 69% less bleeding at the site of the injections than rats that had been injected with saline (see Figure 6).[13] Because the neutrophil is responsible for the majority of the inflammation in an RPA test, the results of this study further support the idea that HIMF affects the function of neutrophils in inflammation.

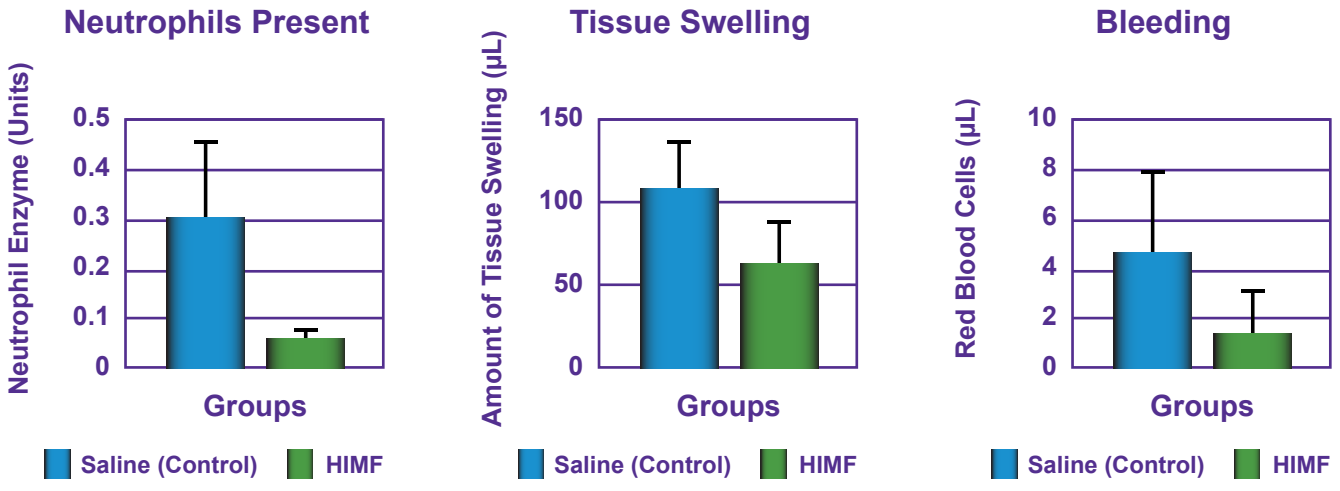

Figure 6. The effect of 20 mg of intravenous (IV) hyperimmune milk factor (HIMF) on the number of neutrophils, amount of tissue swelling, and bleeding seen at the injection sites of a reverse passive Arthus reaction in rats. A significant decrease in neutrophil number, tissue swelling, and bleeding were present ($P < 0.01$, n = 6 per group). Data collected from Beck LR, Fuhrer JP. Anti-inflammatory factor, method of isolation, and use. US Patent #5980953. Nov 1999.

Subcutaneous Sponge Implants

Researchers further tested the effects of HIMF on neutrophils by surgically implanting polyurethane sponges subcutaneously in rats. As foreign material, these SQ sponges trigger inflammation. After the sponges were removed, the number of neutrophils and the volume of edema were measured.[6,13]

In one study, researchers implanted bacteria-free sponges subcutaneously of rats that were simultaneously given 5, 10, 20, or 40 mg of IV HIMF. After their removal, the sponges from rats receiving the 20 and 40 mg doses contained dramatically fewer neutrophils and a mildly less edema than sponges from the rats that did not receive medications (see Figure 7). The rats given 5 or 10 mg did not show a significant difference from the control group. The number of neutrophils was also significantly decreased when HIMF was given 30, 60, and 120 minutes after the implant.[6,13] These studies indicate that HIMF decreases neutrophil migration and edema both prior to and after inflammation ensues.

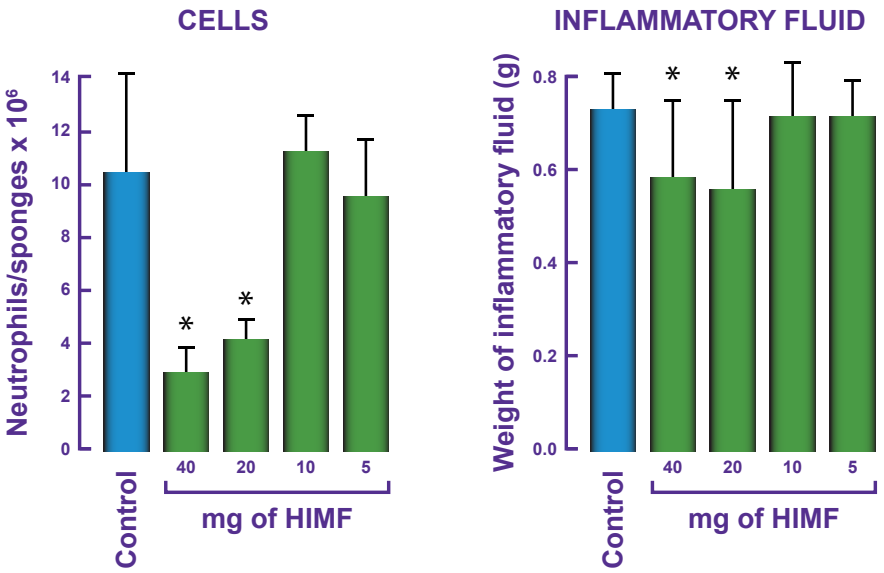

Figure 7. The effect of hyperimmune milk factor (HIMF) on neutrophil migration and fluid accumulation in subcutaneous sponges. * = $P < 0.01$, n = 6 Graphs borrowed from Ormrod DJ, Miller TE. A low molecular weight component derived from the milk of hyperimmunized cows suppresses inflammation by inhibiting neutrophil emigration. *Agents and Actions.* 1992;37:73. Figure5.

Treatments that suppress neutrophil migration are not always indicated in bacterial infections because neutrophils may be necessary to limit bacterial growth. The large bacterial load that can occur after the use of anti-inflammatory medications (such as cyclosporine and methylprednisolone) can cause a rebound effect when massive numbers of neutrophils then rush to the site. This exaggerated rebound response often creates excessive tissue damage and scarring. To see if HIMF is appropriate in patients with bacterial infections, researchers infiltrated SQ sponges with live *Escherichia coli* bacteria. The sponges of rats treated with 40 mg of IV HIMF showed significantly fewer neutrophils but more bacteria than the control group; the decreased number of neutrophils allowed the *E. coli* bacteria to grow unchecked. However, the rebound effect of tissue damage and scarring that can occur with anti-inflammatory drugs did not occur after HIMF testing. Therefore, HIMF is thought to reduce neutrophil migration without causing rebound tissue damage.[4,6,15]

Experimental Pyelonephritis

Scientists experimentally caused pyelonephritis in 26 rats by injecting *E. coli* into their kidneys. Half of the rats were given 40 mg of IV HIMF at the time of the kidney injections and then again 48 hours later. The other half served as the control group. The kidneys were then examined 4 or 21 days later to determine how much fibrosis, edema, and bacteria were present.

After 4 days, the kidneys of the HIMF-treated rats contained higher bacterial counts but 22% less edema accumulation and 24% less scarring than the control group. HIMF was able to not only inhibit inflammation but was able to inhibit tissue damage. By 21 days, however, the kidneys of both the HIMF-treated and control groups showed similar amounts of scarring, edema, and bacteria counts; however, only two doses of HIMF were given early on in the experiment. Similar to the SQ sponge studies, the rebound effect of increased tissue destruction after the use of anti-inflammatory drugs was not seen at this later date.

Intravital Microscopy

To understand how HIMF decreases the number of neutrophils that accumulate at an inflamed site, researchers utilized intravital microscopy to watch neutrophils function in real-time. Woodman et al. used the intravital microscopes to study inflammation induced by platelet-activating factor (PAF), which causes a six-fold increase in neutrophil attachment. Rats that received 40 mg of IV HIMF showed 80-90% less neutrophil attachment in PAF-treated tissues than rats that did not receive HIMF. HIMF also appeared to completely block neutrophils from passing through blood-vessel walls in response to PAF, whereas the rats untreated with HIMF showed a 12-time increase.[6,16]

Importantly, HIMF reversed neutrophil adhesion caused by PAF. The researchers administered PAF then waited 30 minutes before giving 40 mgs of HIMF; this delay allowed the region to become inflamed and neutrophils to attach. Ten minutes after the HIMF was given, there was a significant reduction in the number of neutrophils adhered to the blood vessel wall (see Figure 8). That is, the HIMF appeared to "peel" the neutrophils off of the vessel walls.[6,16] If neutrophils cannot migrate into affected tissue, they cannot participate in or amplify the inflammatory response.

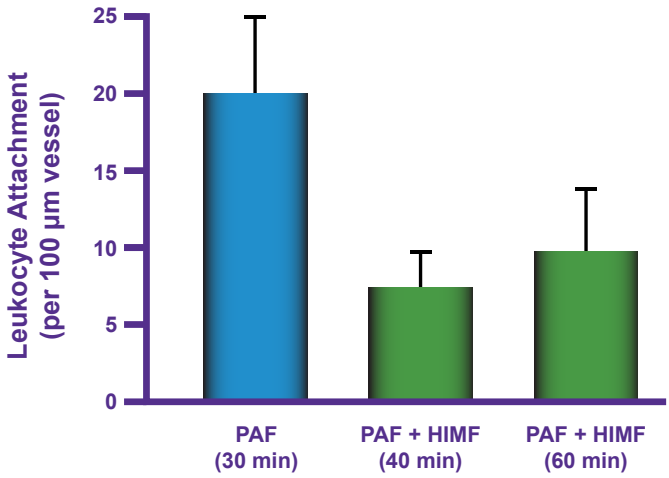

Figure 8. The effect of platelet-activating factor (PAF) and 40 mg of hyperimmune milk factor (HIMF) on the number of neutrophils attached to 100 µm vessel wall. The vessel was treated with PAF alone for 30 minutes. After 30 minutes, HIMF was added. Ten minutes after HIMF was added, there was a large reduction in the number of attached neutrophils. Graph adapted from Beck LR, Fuhrer JP. Anti-inflammatory factor, method of isolation, and use. US Patent #5980953. Nov 1999, Figure 27A.

Flow Cytometry

Flow cytometry detects the microscopic activity of cells. Among other things, it can help measure the activity of receptors on a cell's surface. Woodman et al. used this technology to observe that neutrophils treated with HIMF exhibited fewer CD18 cell receptor proteins than untreated neutrophils. The CD18 protein is part of the activated integrin receptor on neutrophils that firmly binds to a matching ICAM receptor on endothelial cells. When neutrophils express fewer CD18 proteins, they cannot firmly adhere to the endothelium. By inhibiting the CD18 protein, HIMF inhibits the firm attachment stage of neutrophil migration.[6,16]

Transepithelial Electrical Resistance (TER)

Researchers can assess the permeability of tight junctions through the use of transepithelial electrical resistance (TER). Cells with intact tight junctions are polarized. As such, they show a significant difference in electrical charge from one end of the cell to the other. The ability for tight junctions to block an electrical current from travelling from one side to the other (i.e., the ability to maintain the cell's polarization) is termed TER. When tight junctions fully open, the TER is lost.[17]

Stelwagen and Ormrod evaluated the effect of HIMF on tight junction permeability by measuring the TER of mammary and kidney cells. They treated these cells with EGTA, a chemical that opens tight junctions. The cells treated with HIMF maintained their TER significantly better than those that were not treated (see Figure 9). Also, the cells that had been treated with HIMF recovered their TER faster than the control cells (see Figure 9). This *in vitro* study shows that HIMF can protect tight junctions. Although the tight junctions between endothelial cells are considered relatively "loose," the strengthening of endothelial tight junctions could partially account for the reduction in neutrophil migration exhibited by HIMF. In addition, to access certain tissues, such as lung or intestinal tissue, neutrophils must cross tighter epithelial tight junctions.[18]

An additional experiment by Stelwagen and Ormrod showed that HIMF increased the TER value of mammary cells, which indicates that HIMF can actually stimulate the production and strength of tight junctions (see Figure 10). Because tight junctions form in mature cells that are not multiplying, Stelwagen and Ormrod tested HIMF's effect on cell growth. The researchers added HIMF to cultures of multiplying cells; the HIMF-treated cells multiplied less than untreated cells. Because HIMF inhibits cell multiplication, the effects on TER must be caused by "tighter" tight junctions and not because of an increased number of cells.[18]

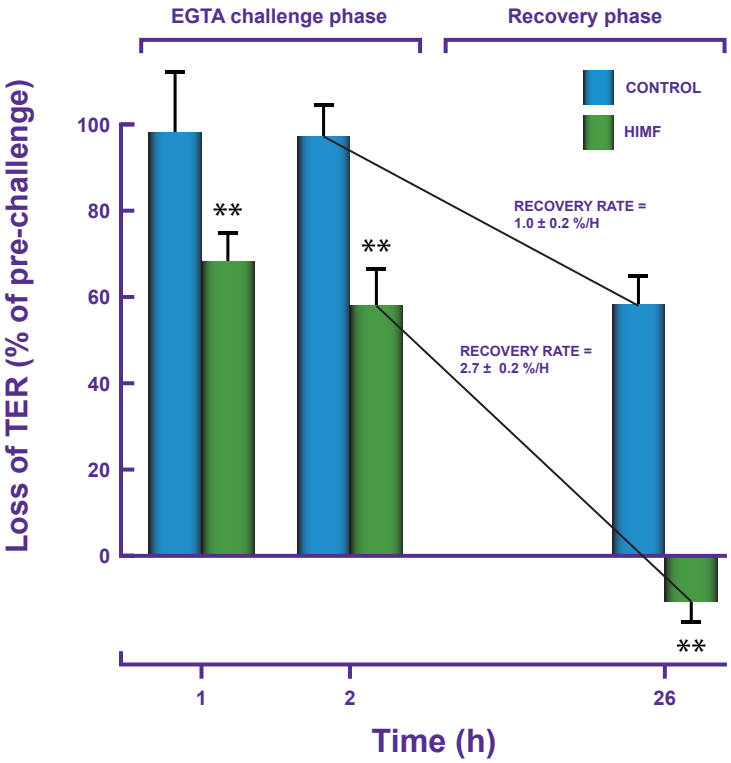

Figure 9. The effect of hyperimmune milk factor (HIMF) on transepithelial electrical resistance (TER)—an indicator of intact tight junctions. Tight junctions open under the influence of EGTA. HIMF prevented the loss of TER significantly more than the untreated control group. Also, the HIMF-treated cells recovered their TER faster than the control group. ** = $P < 0.01$ Graph borrowed from Stelwagen K, Ormrod DJ. An anti-inflammatory component derived from milk of hyperimmunized cows reduces tight junction permeability in vitro. *Inflammation Research.* 1998; 47: 384-388.

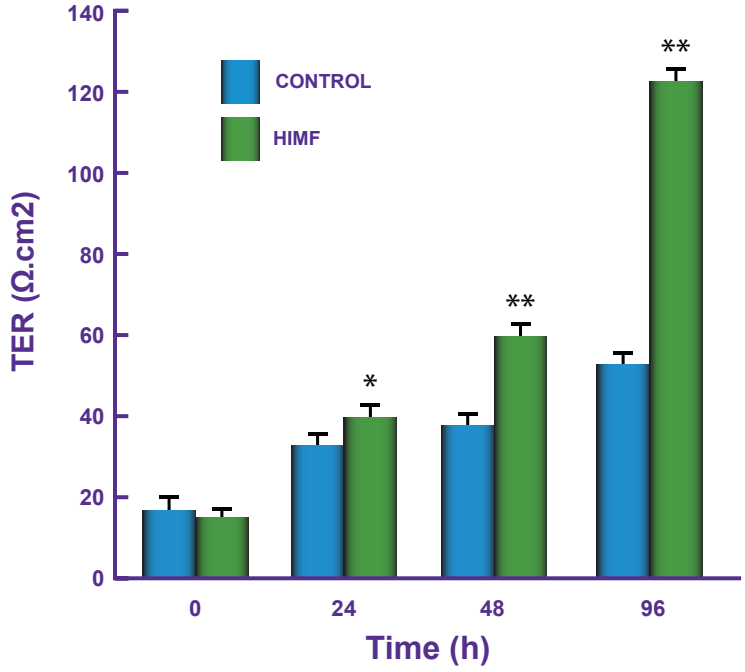

Figure 10. The effect of hyperimmune milk factor (HIMF) on the formation of transepithelial electrical resistance (TER). Mammary epithelial cells were cultured with or without the presence of 2 mg/mL HIMF. * = $P < 0.05$, ** = $P < 0.01$ Graph borrowed from Stelwagen K, Ormrod DJ. An anti-inflammatory component derived from milk of hyperimmunized cows reduces tight junction permeability in vitro. *Inflammation Research.* 1998; 47: 384-388.

Mammary Gland Tests

Owens et al.[19] tested the effects of HIMF on mastitis in both mice and cows. In one study, they infected mice with large amounts of *Staphylococcus aureus* Newbould 305 in order to determine the bacteria's lethal dose or LD_{50}. HIMF provided a protective effect in that the lethal dose of *S. aureus* was significantly higher in mice that had been pretreated with 100 mg/kg HIMF IP for seven days prior to becoming infected. HIMF significantly increased the LD_{50} *S. aureus* 305 from 5×10^9 (untreated) to more than 2×10^{10} CFU in HIMF-treated mice.

Other studies[19] by Owens et al. also suggest a protective effect of HIMF. The researchers infused HIMF directly into the mammary glands of mice before infecting the glands with *S. aureus.* Glands treated with HIMF actually resisted the infection in three of the four mice (75%), while all of the glands of the control group became infected. The glands treated with HIMF also showed fewer inflammatory cells than the control group. Owens et al. also gave IP injection of HIMF for seven days before inducing mastitis with *S. aureus*. The untreated group showed 10 times more bacteria than the HIMF-treated mice. The HIMF-treated mice also showed fewer inflammatory cells and a larger percentage of mammary ducts than the control group (see Figure 11). These mice could therefore secrete larger amounts of milk than the control group.

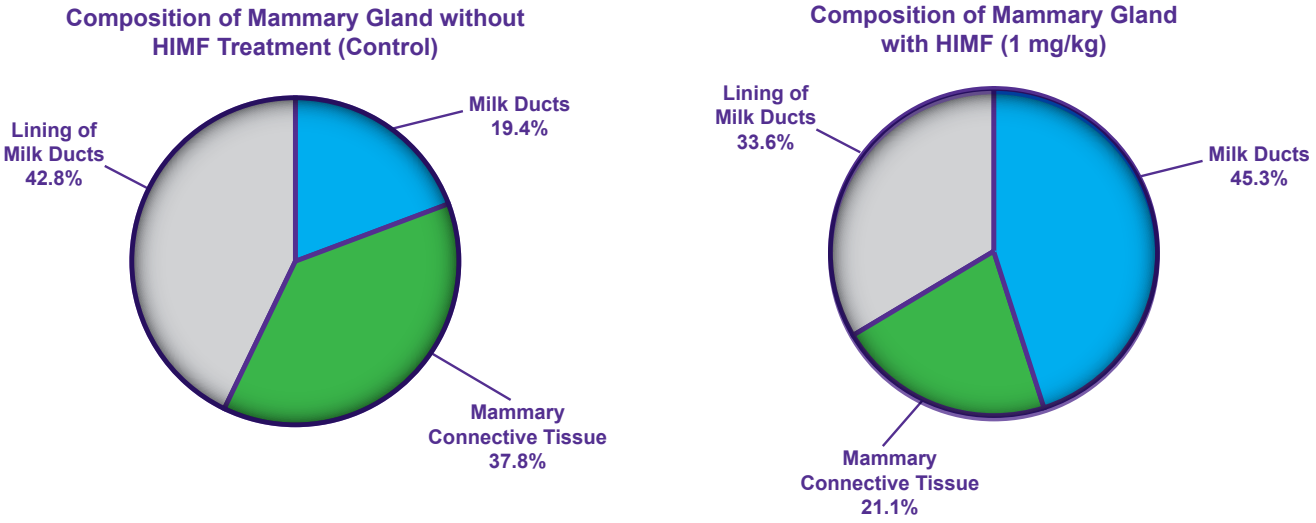

Figure 11. The effect of IP hyperimmune milk factor (HIMF) on the microscopic composition of the mammary gland of mice. The mammary glands of HIMF-treated mice showed a larger percentage of milk ducts (blue portion), which would allow these mice to produce more milk than the control group. All values: $P < 0.05$ Chart data obtained from Owens WE, Nickerson, SC, Washburn PJ. Effect of a milk-derived factor on the inflammatory response to *Staphylococcus aureus* intramammary infection. *Veterinary Immunology.* 1992; 30: 233-246.

To further understand the protection provided by HIMF in bacterial infections, Owens et al.[19] tested the function of macrophages. Opsonized *S. aureus* bacteria were added to cultures of cow mammary gland macrophages. After four hours, the number of bacteria phagocytized by macrophages were counted. Macrophages treated with HIMF phagocytized 10 times more bacteria (1430 CFU) than untreated macrophages (135 CFU) (see Figure 12). HIMF, therefore, appears to increase the ability of macrophages to destroy bacteria.

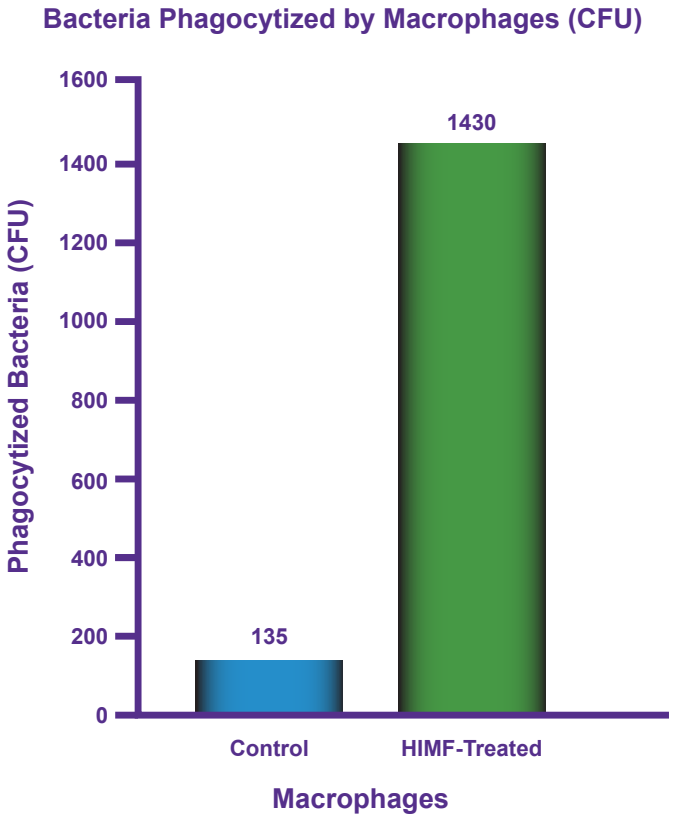

Figure 12. The effect of 2000 µg/mL hyperimmune milk factor (HIMF) on the ability for macrophages to engulf bacteria. The results indicate that HIMF increases the ability of macrophages to destroy bacteria in vitro. Graph data obtained from Owens WE, Nickerson, SC, Washburn PJ. Effect of a milk-derived factor on the inflammatory response to *Staphylococcus aureus* intramammary infection. *Veterinary Immunology.* 1992; 30: 233-246.

In another experiment, Owens et al.[19] tested the effects of HIMF on mastitis in cows by introducing *S. aureus* into their udders. Udders infused with 5 mg of HIMF 24 hours before the introduction showed fewer infections (5 out of 10) than udders treated with saline (7 out of 10). Milk from HIMF-treated udders also showed fewer bacteria than the milk from saline-treated udders during the first three days following the infections. However, the HIMF did not eliminate the infection.

Host-versus-Graft and Graft-versus-Host Assays

Researchers utilize host-versus-graft assays in order to test the function of lymphocytes. They inject a rat with lymphocytes from its F1 hybrid offspring. Because these lymphocytes (graft) appear foreign to the parent (host), the parent's T-lymphocytes target them. The resultant lymph node enlargement can be weighed to quantify the magnitude of the immune response.

Oppositely, grafts can attack the host. These immune cells see their new body as non-self and target the recipient. Researchers replicate this process in the graft-versus-host assay by injecting the parent's lymphocytes into its F1 hybrid offspring.

In a host-versus-graft assay, Ormrod and Miller[15] gave 20 mg of IV HIMF to host rats 48, 24, and 3 hours prior to injecting lymphocytes from their untreated F1 offspring. HIMF suppressed the host-versus-graft reaction by 30%. In a graft-versus-host assay, Ormrod and Miller[15] injected HIMF-treated rat host lymphocytes into their F1 hybrid offspring. No change in the graft-versus-host reaction was seen. These studies indicate that HIMF affects the function of lymphocytes within the body. The splenic weight and cell count increased after HIMF use, suggesting that HIMF causes lymphocytes to accumulate in the spleen.

Ormrod and Miller[15] also applied Concanavalin-A (which causes lymphocytes to multiply) to extracted lymphocytes in order to increase the number of lymphocytes that could be used in the experiments. Lymphocytes from rats untreated with HIMF multiplied as expected. When the concanavalin-A was applied to lymphocytes from HIMF-treated rats, however, the lymphocytes did not multiply. This finding further supports the idea that HIMF interferes with the ability for lymphocytes to function normally.

Collagen-Induced Arthritis Model in Mice

A common laboratory test for rheumatoid arthritis is the collagen-induced mouse model. In this test, researchers inject type II collagen into the skin of mice. The collagen triggers rheumatoid arthritis symptoms in approximately 21-28 days. Trained technicians then assess the degree of arthritis by scoring the amount of redness, tissue swelling, and joint stiffness exhibited by the mice. The study is blinded so that the technicians do not know which mice receive the medications and which mice are in the control group.[20]

Gingerich et al. (1997)[21] mixed HIMF, Stolle's Immune Milk, a whey protein isolate (WPI Plus), or a salt solution (control) daily into the food of 40 mice. Stolle's Immune Milk, HIMF, and WPI Plus—all of which are made from the milk of hyperimmunized cows—significantly decreased the occurrence and the severity of the arthritis seen (see Figure 13). This study indicates that HIMF has similar anti-inflammatory effects as Stolle's Immune Milk, from which MicroLactin® is derived.

Other studies of Stolle's Immune Milk have showed that it relieves the symptoms of rheumatoid arthritis;[22] prevents the age-related decline of the immune system;[23] prevents certain infections in immunocompromised patients;[23] and reduces atherosclerosis, blood pressure, and cholesterol levels.[7]

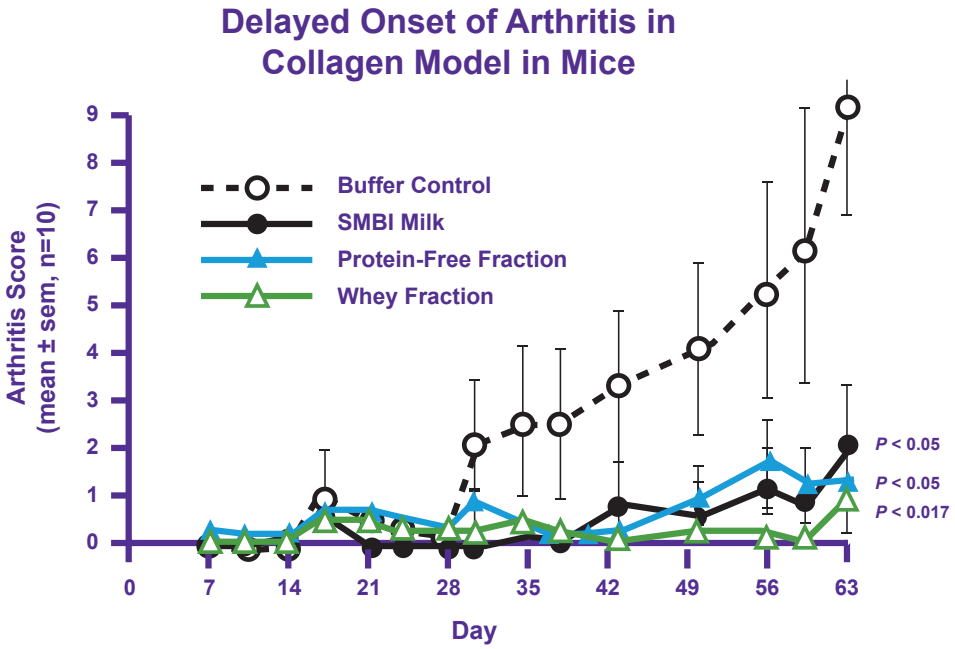

Figure 13. The arthritis score of mice treated with oral salt solution (control), Stolle (SMBI) Immune Milk, protein-free fraction (HIMF), or whey fraction (WPI Plus). The occurrence and severity of arthritis symptoms (redness, tissue swelling, and joint stiffness) were significantly decreased with Stolle milk ($P < 0.05$), HIMF ($P < 0.05$), and WPI Plus ($P = 0.017$). Graph borrowed from Woods C, Gingerich D. VPL Technical Brief: Pharmacology of MicroLactin®.

Rat Lungs Exposed to Cigarette Smoke

One such experiment of Stolle's Immune Milk—which is milk from hyperimmunized cows—studied the effect of daily cigarette smoke on the lungs of rats. Wilborn et al.[7] gave rats either water, normal milk, or Stolle Immune Milk for the duration of the experiment and the two weeks prior to smoke exposure. For two weeks, rats were placed daily inside a cigarette smoke machine, in which they breathed the smoke of 2 cigarettes for 20 minutes per day. The exposure to cigarette smoke caused the rats who drank water or normal milk to develop inflammation around their airways, lung damage, and debris within the lungs. The rats that drank Stolle Immune Milk showed markedly less inflammation and essentially no smoke debris. Compared to the control rats, the macrophages in the rats who drank Stolle Immune Milk showed evidence of greater than 50% more phagocytosis, more lysosomal activity, and greater regeneration. Stolle Immune Milk activated macrophages to work more effectively to eliminate harmful smoke debris, allowing the rats to have clearer airways with easier respiratory function.[7]

References

[1] Tizard, I. Adaptive immunity. *Merck Veterinary Manual* Web site. Accessed July 2, 2017. Available at http://www.merckvetmanual.com/immune-system/the-biology-of-the-immune-system/adaptive-immunity.

[2] Edwards, SH. Chemical mediators of inflammation. *Merck Veterinary Manual* Web site. Accessed July 2, 2017. Available at http://www.merckvetmanual.com/pharmacology/anti-inflammatory-agents/chemical-mediators-of-inflammation.

[3] Muller WA. Getting leukocytes to the site of inflammation. *Veterinary Pathology*. 2013; 50(1): 7-22. Available at https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3628536/

[4] Beck LR, Fuhrer JP. Milk lymphocyte anti-adhesion factor, and its role as an anti-microbial. *International Dairy Federation*. 1993; 62-72.

[5] Allen J, Hector D. Benefits of breastfeeding. *New South Wales Public Health Bulletin*. 2005; 16: 42-46.

[6] Beck LR, Fuhrer JP. Anti-inflammatory factor, method of isolation, and use. US Patent #5980953. Nov 1999.

[7] Wilborn WH, Hyde BM, Beck LR, Fuhrer JP. Milk from hyperimmunized cows stimulates lysosomal activity in rat lung macrophages. Summary of presentation at The Lovelace Respiratory Research Institute Symposium on Respiratory Immunology; Santa Fe, NM. 1999.

[8] Gingerich DA, Strobel JD. Use of client-specific outcome measures to assess treatment effects in geriatric, arthritic dogs: controlled clinical evaluation of a nutraceutical. *Veterinary Therapeutics.* 2003; 4(1): 56-66.

[9] Bello TR, Allen TA. The use of MicroLactin for inflammatory conditions in equine veterinary practice. *Journal of Equine Veterinary Science.* 2005; 25(9): 380-382.

[10] Bello TR, Allen TM. An intensive approach in the treatment of clinical equine protozoal myeloencephalitis. *Journal of Equine Veterinary Science*. 2008; 28(8): 479-483.

[11] MacKay RJ. Equine protozoal myeloencephalitis: treatment, prognosis, and prevention. *Clin Tech Equine Pract*. 2006; 5: 9-16.

[12] Ormrod DJ, Miller TE. The anti-inflammatory activity of a low molecular weight component derived from the milk of hyperimmunized cows. *Agents and Actions*. 1991; 32(3/4): 160-166.

[13] Ormrod DJ, Miller TE. A low molecular weight component derived from the milk of hyperimmunized cows suppresses inflammation by inhibiting neutrophil emigration. *Agents and Actions*. 1992; 37: 70-79.

[14] Beck LR. Method of treating inflammation using bovine milk. US Patent #4284623. Aug 1981.

[15] Ormrod DJ, Miller TE. Milk from hyperimmunized dairy cows as a source of a novel biological response modifier. *Agents and Actions*. 1993; 38(Special Conference Issue): C146-C149.

[16] Woodman R, Fuhrer P, Beck L, Kubes P. The effects of hyperimmunized milk factor (HIMF) on neutrophil adhesion *in vivo* [Abstract]. Society for Leukocyte Biology, 29th National Meeting, Charleston South Carolina. 1992.

[17] Sonoda S, Spee C, Barron E, Ryan SJ, Kannan R, Hinton DR. A protocol for the culture and differentiation of highly polarized human retinal pigment epithelial cells. *Nature protocols*. 2009; 4(5): 662-673.

[18] Stelwagen K, Ormrod DJ. An anti-inflammatory component derived from milk of hyperimmunized cows reduces tight junction permeability in vitro. *Inflammation Research.* 1998; 47: 384-388.

[19] Owens WE, Nickerson SC, Washburn PJ. Effect of a milk-derived factor on the inflammatory response to *Staphylococcus aureus* intramammary infection. Veterinary Immunology and Immunopathology. 1992; 30: 233-246.

[20] Rosloniec EF, Cremer M, Kang AH, Myers LK, Brand DD. Collagen-induced arthritis. *Curr Protoc Immunol.* Apr 2010; Chapter 15: Unit 15.5.1-25.

[21] Gingerich DA, Strobel JD, Brown A, Fuhrer JP. Efficacy of SMBI milk bioactive factors in a collagen-induced arthritis model in mice. Summary of presentation at Japan Rheumatism Association; Tokyo, Japan. 1997.

[22] Stolle RJ, Beck LR. Prevention and treatment of rheumatoid arthritis. US Patent #4732757A. 1991.

[23] Beck LR, Ishida A, Yoshikai Y, Murosaki S, Kubo C, Hidaka Y, Nomoto K. Use of hyperimmune milk to prevent suppression of T-lymphocyte production. US Patent #6056978. May 2000.

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