



## Technical Brief: Pharmacology of Microlactin<sup>®</sup>

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Duralactin<sup>™</sup> contains Microlactin<sup>™</sup>, a patented special milk protein concentrate (SMPC) from the milk of hyperimmunized cows. Partially purified preparations inhibit inflammation in many laboratory animal models. The anti-inflammatory activity is bio-available both orally and systemically, is effective regardless of the etiology of the inflammation, and appears to function with no evidence of gastro-intestinal tract irritation.

Partially purified factors derived from MicroLactin<sup>™</sup> consist of one or more non-steroidal, non-peptide molecules with molecular weights of less than 1,000 daltons. These factors are highly soluble in water at high and low pH and in aqueous organic solvents at low pH. Gas chromatography and mass spectroscopy studies to date have found no evidence of known carbohydrate, lipid, steroid, or peptide structures. Definitive chemical and structural studies of the molecule(s) are in progress.

Results of numerous pharmacologic investigations suggest novel activities as summarized in the following table.

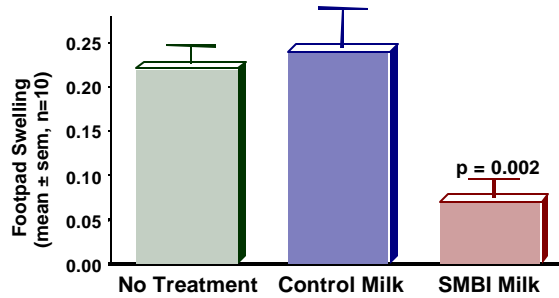
Action	Model	Test material	Reference
activates macrophages	rats exposed to smoke	milk, whey, and filtrate	Wilborn et al
inhibits carrageenan-induced inflammation	rat paw edema test	HIMF*	Ormrod, et al
inhibits immune complex neutrophil response	Arthus reaction, rat	HIMF*	Ormrod, et al
inhibits neutrophil migration	subcutaneous sponge, rat	HIMF*	Ormrod, et al
inhibits infection-induced inflammation	mouse mastitis	HIMF*	Owens, et al
reduces tissue damage in infection	infectious nephritis, rat	HIMF*	Beck, Ormrod
inhibits neutrophil adhesion	PAF-induced adhesion, rat	HIMF*	Woodman, Kubes
reduces tight junction permeability	<i>in vitro</i> epithelial cells	HIMF*	Stelwagen, Ormrod
inhibits arthritis	collagen arthritis, mice	MDF*, milk, WPI*	SMBI
inhibits TPA-induced inflammation	mouse ear swelling	milk, whey, MicroLactin	SMBI
inhibits autoimmune disease	MRL autoimmune mouse	milk, whey, MicroLactin	Murosaki, SMBI

\* defined in text

### *Rat paw edema tests*

The presence of anti-inflammatory activity in milk and methods of enhancing its expression were first disclosed and patented in 1981. The activity was demonstrated in milk by the standard rat paw edema assay (Beck, 1981). Briefly, rats were fed milk from either hyperimmunized or non-immunized cows for five days before carrageenan injection into the footpad. Significant ( $p < 0.01$ ) suppression of edema was demonstrated in hyperimmune milk-fed rats but not controls.

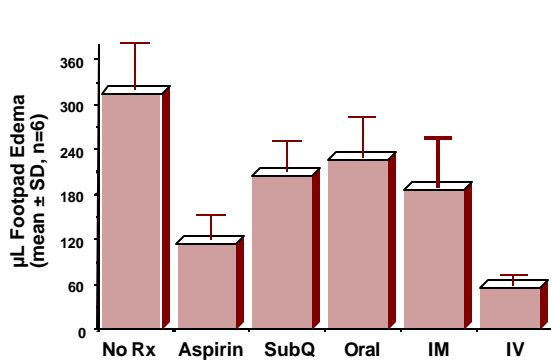
### Anti-inflammatory Effects in Paw Swelling Test (Beck, Patent)



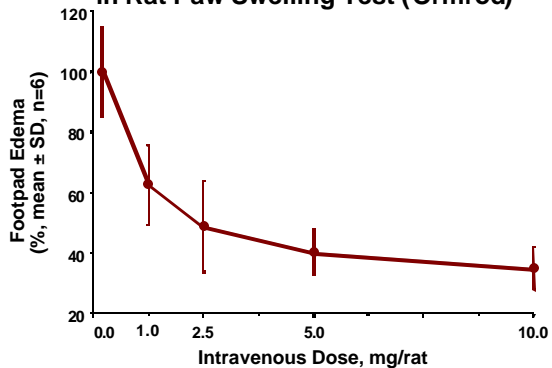
Fractionation of the milk by ultrafiltration revealed that anti-inflammatory activity is expressed in the dialyzable, <10,000 dalton fraction (Beck, 1988). A partially purified anti-inflammatory factor (AIF), was subsequently prepared according to published methods. Briefly, immune whey permeate was concentrated 5X, applied to a Sephadex G-10 column and eluted with water, and monitored at 254 and 280nm. The first peak eluted from the G10 column was found to be bioactive in the rat paw bioassay and was collected and lyophilized for further pharmacologic evaluation.

Ormrod and Miller (1991, 1992, 1993) undertook systematic studies of the biologic activity of the partially purified preparation at Auckland University Hospital in New Zealand and coined the term "HIMF", an acronym for hyperimmune milk factor. Details of experimental procedures and results are published elsewhere. HIMF administration inhibited paw edema in rats in response to carrageenan injection. This activity was demonstrated by subcutaneous, intraperitoneal, intramuscular, and intravenous injection of HIMF as well as oral administration. The anti-inflammatory effect of HIMF was particularly striking following intravenous infusion. Results of a typical assay are presented in the following figures.

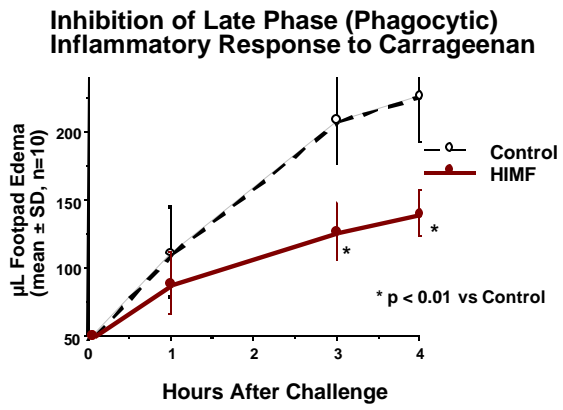
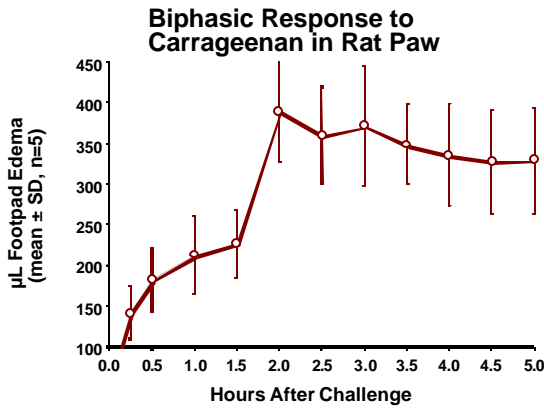
### HIMF Inhibits Footpad Swelling



### Dose-response Curve of HIMF in Rat Paw Swelling Test (Ormrod)



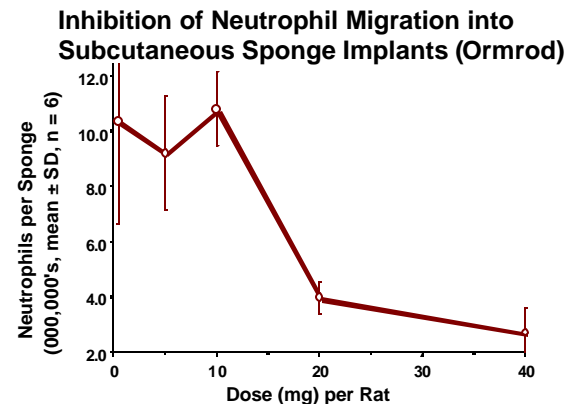
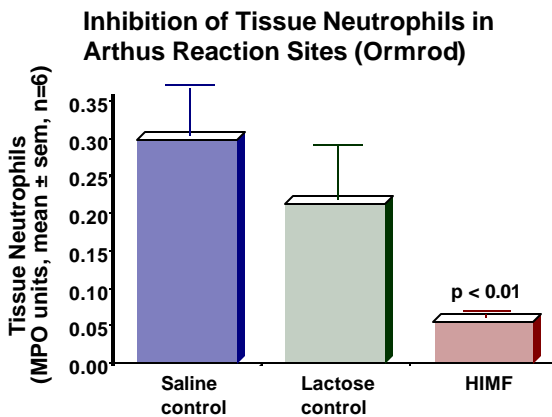
It is well known that the inflammatory response to carrageenan is biphasic as demonstrated through sequential determinations of footpad edema in the present model in rats. The early, non-cellular phase is initiated by soluble mediators including histamine and bradykinin, while the later phase is mediated by the activities of inflammatory cells, chiefly neutrophils. In order to gain insight into the mechanism of action, the response to carrageenan injection was monitored at frequent intervals to determine whether the inhibition occurred early or late. The early non-phagocytic phase was unaffected by intravenous administration of HIMF whereas the secondary (cellular) phase was significantly ( $p < 0.01$ ) inhibited as illustrated in the following figures.



These findings suggested that the anti-inflammatory effect may be due to an effect on inflammatory cells, likely the neutrophils.

### Effect on neutrophil migration

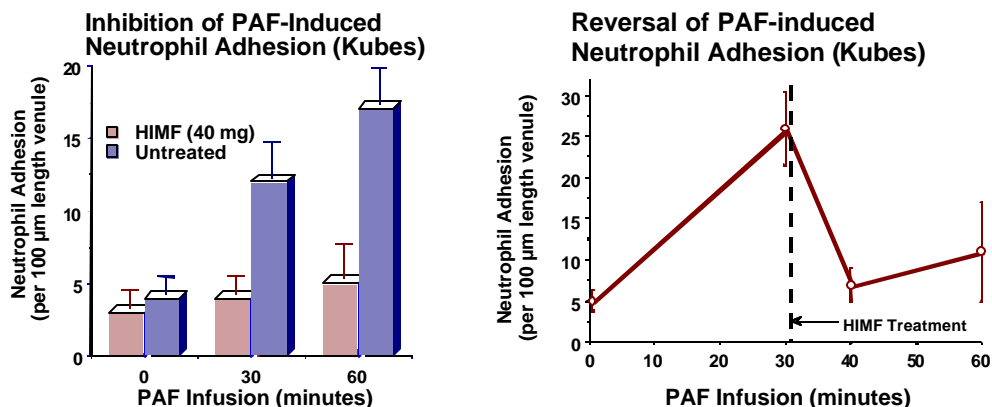
To further elucidate the effects of the factor(s) on cellular function, additional experiments were conducted using other models of inflammation. In the Arthus reaction model in the rat, intravenous injection of HIMF at the time of challenge inhibited accumulation of edema and hemorrhage and markedly suppressed neutrophil accumulation. Intravenous injection of HIMF also dose-dependently suppressed the accumulation of neutrophils in subcutaneously implanted sponges in rats.



The above findings collectively support the hypothesis that the anti-inflammatory effects are due to inhibition of neutrophil participation in the inflammatory response.

### Effect on neutrophil adhesion

To determine the nature of the suppression of neutrophil participation, the effect of HIMF on neutrophil adhesion and emigration from single inflamed post-capillary venules was investigated by intravital microscopy in anesthetized rats (Woodman et al, 1992). HIMF blocked platelet activating factor- (PAF) induced binding of neutrophils to the endothelial capillary walls in a dose-dependent manner. Importantly, HIMF treatment also reversed neutrophil adhesion to the capillary endothelial cells.



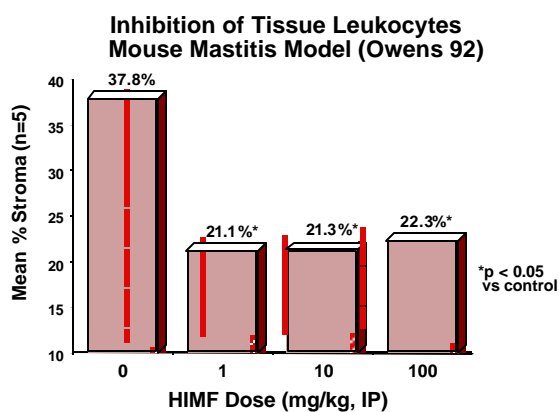
These results are potentially important pharmacologically because receptor-dependent neutrophil adhesion is a rate-limiting step associated with tissue injury in a number of inflammatory conditions.

### Effect on tight junctions

Stelwagen and Ormrod (1998) provided evidence suggesting that AIF, as expressed in HIMF, may act by promoting tight junction formation, preventing loss of tight junction integrity during challenge, and promote post challenge recovery. SMBI scientists recently found that exposure of endothelial cells to MPC upregulates expression of the gene for ZO-1, a tight junction regulatory protein. The anti-inflammatory properties of the milk factors could, therefore, be mediated by restricting the transmigration of leukocytes through tight junctions (diapedesis).

### Tissue-sparing during infection

Because bioactive factors in MicroLactin™ originate from milk, it is tempting to speculate that their teleologic purpose might include protection of the mammary gland itself. To pursue this possibility experiments were conducted in *Staphylococcus aureus*-induced mastitis in mice (Owens, Nickerson, and Washburn, 1992). The LD<sub>50</sub> of *S aureus* challenge inoculum was increased in mice given daily intraperitoneal injection of HIMF at 100 mg/kg for 7 days prior to intramammary challenge. Pretreatment with HIMF at dosages of 1, 10, and 100 mg/kg also resulted in significantly greater percentages of alveolar lumen and less stroma and leukocyte infiltration 24 hours after infection.

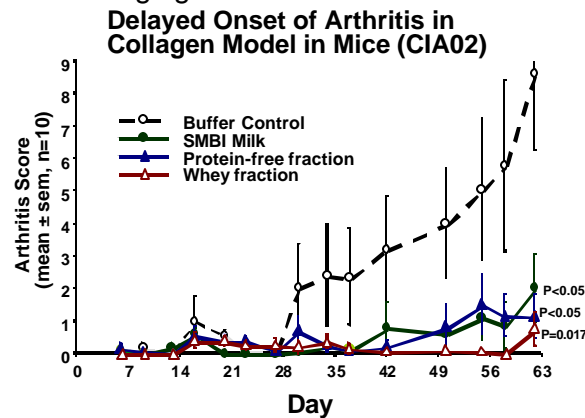


The results of these and other findings in the model led the authors to conclude that HIMF induces nonspecific enhancement of host defenses against bacterial challenge as well as anti-inflammatory activity.

In another model, pyelonephritis was induced by direct inoculation of the kidneys of rats with live *E. coli*. Following infection, bacterial numbers increased rapidly in both HIMF-treated and control rats to reach a peak 3 to 4 days later. However, kidney weights and surface lesions in HIMF treated rats were 22 and 24 percent lower than in controls. This indicated that HIMF treatment had no suppressive effect on bacterial growth but did mediate a marked anti-inflammatory, tissue sparing effect in the face of infection (Beck and Fuhrer, 1995).

### Collagen-induced arthritis in mice

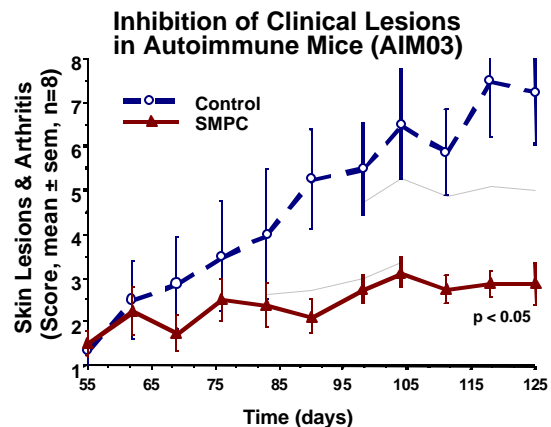
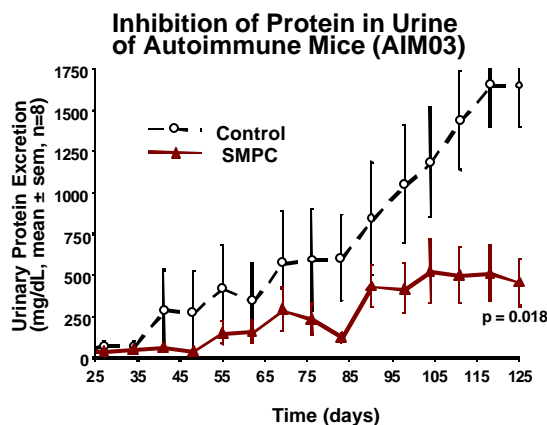
SMBI scientists conducted feeding studies in this animal model of human rheumatoid arthritis to determine whether the putative efficacy of SMBI Milk in arthritis could be attributed to its anti-inflammatory activity as expressed in protein-free fractions. Intact SMBI milk, whey protein isolate made from that milk, and the protein-free fraction all significantly ( $p < 0.05$ ) inhibited arthritis as illustrated in the following figure.



These results were presented and published as an abstract at the Japan Rheumatology Association (Gingerich, 1997).

### Laboratory animal studies on MicroLactin<sup>Ô</sup>

MicroLactin<sup>TM</sup> was tested orally in the rat paw edema and in the TPA-induced mouse ear swelling models and found to express acute anti-inflammatory activity (data not shown). Long term feeding studies were also conducted in the autoimmune-prone, MRL mouse model of rheumatoid arthritis and systemic lupus erythematosus. MicroLactin<sup>TM</sup> feeding delayed the increase in rheumatoid factor titer and the onset of proteinuria, which are hallmarks of autoimmune disease, and prevented the development of characteristic skin lesions and signs of arthritis.



## **Conclusion**

On the basis of results of laboratory animal tests described above, it was concluded that the bioactive factors in milk from hyperimmunized cows have been captured in MicroLactin™. Double-blind controlled clinical trials have now been conducted in human patients with osteoarthritis. The results, which confirmed the clinical relevance of the pharmacological findings, are published in peer-reviewed medical journals. A double-blind, controlled clinical trial of MicroLactin™ supplementation in older, large breed dogs was also conducted and the results confirmed the clinical value of the immunonutritional approach to management of musculoskeletal inflammatory disorders in dogs.

## REFERENCES

- Beck, LR. (1981, Aug 18) Method of treating inflammation using bovine milk, US Patent #4,284,623.
- Beck, LR. (1990, Sep 11) Anti-inflammatory factor, method of isolation, and use, US Patent #4,956,349.
- Beck, LR & Fuhrer JP (1999, Nov 9) Anti-inflammatory factor, method of isolation, and use, US Patent #5,980,953
- Beck, LR and Fuhrer, PF. (1994) Milk lymphocyte anti-adhesion factor and its role as an anti-microbial. *Proceedings Indigenous Antimicrobial Agents of Milk - Recent Developments*. Uppsala: Int. Dairy Fed. 62-72.
- Gingerich, DA (1997) Efficacy of immune milk bioactive factors in the collagen-induced arthritis model in mice. Tokyo: Japan Rheumatism Association.
- Goldman, AS et al. (1986) Anti-inflammatory properties of human milk. *Acta Paediatr Scand*, **75**, 689-695.
- Ormrod, DJ and Miller TE. (1991) The Anti-inflammatory activity of a low molecular weight component derived from the milk of hyperimmunized cows. *Agents and Actions*, **32**, 160-166.
- Ormrod, DJ and Miller TE. (1992) A low molecular weight component derived from the milk of hyperimmunized cows suppresses inflammation by inhibiting neutrophil emigration. *Agents and Actions*, **35**, 1-10.
- Ormrod, DJ and Miller TE. (1993) Milk from hyperimmunized dairy cows as a source of a novel biological response modifier. *Agents and Actions*, Special Conference Issue.
- Owens, WE, Nickerson, SC and Washburn, PJ. (1992) Effect of a milk-derived factor on the inflammatory response to *Staphylococcus aureus* intramammary infection. *Vet Immunol & Immupath*, **30**, 233-246.
- Stelwagen, K and Ormrod, DJ: (1998) An anti-inflammatory component derived from milk of hyperimmunized cows reduces tight junction permeability in vitro. *Inflamm res*, **47**, 384-388.
- Tetens J: (2001) Gastrointestinal mucosal permeability – role of the tight junction. *Proc 19<sup>th</sup> ACVIM*, Denver, 188-189.
- Wilborn, WH, Hyde, BM, Beck, LR and Fuhrer, JP. (1999) Milk from Hyperimmunized Cows Stimulates Lysosomal Activity in Rat Lung Macrophages. *Abstract*. The 1999 Lovelace Respiratory Research Institute Symposium on Respiratory Immunology, Santa Fe, NM, Oct. 10 - 13, 1999
- Williams, TJ, Hellewell, PG and Jose, PJ. (1986) Inflammatory mechanisms in the Arthus reaction. *Agents and Actions*, **19**, 66-72.
- Woodman, R., Fuhrer, P., Beck, L. and Kubes, P. The effects of hyperimmunised milk factor (HIMF) on neutrophil adhesion in vivo. Abstract, Society For Leukocyte Biology, 29th National Meeting, Charleston, South Carolina, USA (1992).