

## INTERFERON ACTIVITY IN BOVINE COLOSTRUM AND MILK

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### Introduction

Studies have shown that IFNs have potent immunologic (1,2) and antiviral (3,4) activity in both in vitro and in vivo testing in the bovine species. In calves given IFN orally, enteric disease due to rotavirus or mixed rotavirus and coronavirus infection was prevented or reduced (5). Interferons are also involved in immunity to protozoal infections (6), and a polypeptide with many characteristics similar to an alpha IFN has been identified that stimulated macrophages to inhibit intracellular development of Eimeria bovis (7). In preliminary studies, calves administered partially purified natural bovine alpha IFN intravenously had less coccidial diarrhea than control calves (8).

Although lymphocytes isolated from human colostrum and milk are capable of producing interferon (IFN) when stimulated in vitro (9,10), assays of human colostrum and milk for IFN have usually yielded negative results (9-11). Since bovine peripheral blood lymphocytes, when stimulated with virus, are capable of producing alpha IFN (12), it would seem logical that these cells in mammary secretions would also have this ability. The only citation of the spontaneous occurrence of IFN in milk or colostrum from any species is a brief mention that a sample from one woman's breast milk contained antiviral activity (11). It was not known whether this woman was infected with a virus (11). It has been well documented, however, that when mother mice were inoculated with Newcastle disease virus, their milk contained measurable IFN (7). Furthermore, the newborns suckling such mothers had a significantly greater survival rate than that of controls after lethal challenge with vesicular stomatitis virus (7).

Since most mammalian infants have a lower incidence of neonatal disease when raised on maternal milk as opposed to milk replacer (13,14), it was hypothesized that interferons in mammary secretions may play an important role in neonatal immunity. Since bovine milk and colostrum had not been heretofore examined, the objective of this study was to determine whether IFN could be found in detectable levels in normal bovine colostrum and milk.

### Materials and Methods

#### Detection of IFN in bovine milk and colostrum:

A 600 ml sample of milk from a healthy cow from the University

of Georgia Dairy was initially used. The cow was one month post-partem. The sample was defatted by several centrifugations at 10,000 Xg for 30 min (4°C). This resulted in a tri-layered sample (fat on top, fluid milk in middle, pelleted cells at bottom). Fat and pelleted somatic cells were discarded. The milk was then filtered multiple times: first through Whatman filter paper number 1, through 8 micron, 1 micron, 0.45 micron filters, and last through a 0.22 micron sterilization filter. This process proved to be extremely laborious. Since low concentrations of IFN were anticipated, the milk was concentrated to a final volume of 100 ml (6X concentration) by Amicon spiral membrane centrifugation prior to IFN assay. A sample of bovine colostrum measuring in the "green" (adequate immunoglobulin) range by Colostrometer" was subsequently processed and assayed unconcentrated.

#### Characterization of the antiviral substance as IFN:

Samples of milk and colostrum processed as described above were subjected to acid, trypsin, and heat treatments as follows: A first set of samples was lowered to pH 2 with 2N HCl, refrigerated over night, and the pH was then adjusted to 7.4 with 2N NaOH. A second set of samples was mixed with equal volumes of 0.05% EDTA trypsin and incubated at 37°C for 1 hour. Then cold fetal bovine serum was added to inactivate the trypsin. A third set of samples was held at 56°C in a heat block for 15 min. All samples were assayed for IFN activity before and after treatments.

### Results and Discussion

The antiviral activity in milk was pH 2.0 stable and that in milk and colostrum was both trypsin and heat labile, conforming to normally accepted standards for alpha and beta IFNs. In the colostrum, approximately 50% of the original activity remained after acid treatment, suggesting the presence of both alpha (or beta) and gamma interferons.

An unexpected finding was the detection of IFN in 6X concentrated milk at 160 U/ml (ie, approximately 30 U/ml undiluted) and in unconcentrated "green" colostrum at very high levels (5,120 U/ml). In other studies, investigators were unable to detect IFN in human breast milk with the exception of a sample from 1 woman for whom the virus infection status was unknown (11). Although it is well documented that leukocytes isolated from breast milk can produce IFN when stimulated with virus, the only other report of the spontaneous presence of IFN in milk is that of IFN in milk from mother mice experimentally inoculated with virus (7). These data, together with the extremely low doses of IFN reported to be immunologically active orally (1-10 U/lb) (15) led us to expect a much lower titer of IFN in mammary secretions. Although many samples must be tested before a true "physiologic level" of IFN in bovine milk is determined, some extrapolations can be made from our preliminary data: A 100 lb Holstein calf drinking 4 liters of whole bovine milk/day (normal amount) would be receiving 1,200 U/lb of IFN if the milk contains 30 U/ml. It will also be important to do serum Ab screen for common viral infections on the cows from which milk and colostrum are taken.

A second unexpected finding was the apparent presence of another substance in both the milk and colostrum samples that was inhibiting IFN activity. As the samples were diluted across the row of the microtiter plate, the cytotoxicity decreased and the IFN activity increased (instead of becoming weaker) up to a point of maximal antiviral activity. Since control wells with milk or colostrum, but without virus showed no cytotoxicity, the substance did not appear to be directly cytotoxic to the assay cells, but instead appeared to be inhibiting the antiviral action of IFN present in the samples. We intend to pursue investigations as to the possible nature of this cytotoxic or inhibitory substance. Results of one preliminary assay suggested that this substance was acid labile, which would indicate that it is not likely to be TGF beta.

### Summary

Antiviral activity was detected in one sample of bovine milk and one sample of colostrum, both collected from clinically healthy mature cows. In milk, this activity was pH 2.0 stable and trypsin and heat labile, conforming to normally accepted standards for alpha and beta IFNs. In the colostrum, approximately 50% of the original activity remained after acid treatment, suggesting the presence of both alpha and gamma interferons.

The level of IFN in milk was approximately 30 U/ml and that of colostrum was 5,120 U/ml. An unexpected finding was the presence of an acid-labile substance in both the milk and colostrum samples that inhibited IFN activity. This warrants further investigation.

The findings of this study must be considered preliminary, since the virus infection status of the cows sampled was unknown. Larger numbers of samples will be necessary to determine a normal physiologic level of IFN in bovine colostrum and milk.

### References

1. Bielefeldt-Ohmann, H., Babiuk, L.A., Alteration of some leukocyte functions following in vivo and in vitro exposure to recombinant bovine alpha- and gamma-interferon. *J. Interferon Res.* 6: 123-136. 1986.
2. Roth, J.A., Frank, D.E., Recombinant bovine interferon-gamma as an immunomodulator in dexamethasone-treated and nontreated cattle. *J. Interferon Res.* 9: 143-151. 1989.
3. Cummins, J.M., Hutcheson, D.P., Low dosage of interferon to enhance vaccine efficiency in cattle. *Proc 18th Annu. Meeting AABP* 18: 135-138. 1985.
4. Babiuk, L.A., Bielefeldt-Ohmann, H., Gifford, G., Czarniecki, C.W., Scialli, V.T., Hamilton, E.B., Effect of bovine alpha 1 interferon on bovine herpesvirus type 1-induced respiratory disease. *J. Gen. Virol.* 66: 2383-2394. 1985.
5. Hofmann, V.W., Danna, K., Seeger, K., Erste Erfahrungen bei der Behandlung von virus bedingten Kalberdurchfallen mit gentechnisch erzeugtem interferon. *Deutsch Tierarztl. Wschr.* 92: 245-300. 1985.
6. Sonnenfeld, G., Degee, A.L.W., Mansfield, J.M., Newsome, A., Arnold, R.R., Kierszenbaum, F. Role of interferon in resistance and immunity to protozoa. *The Biol. of the IFN System*, 1984.
7. Speer, C.A.,

Reduker, D.W., Burgess, D.E., Whitmire, W.M., Splitter, G.A., Lymphokine-induced inhibition of growth of *Eimeria bovis* and *Eimeria papillata* (Apicomplexa) in cultured bovine monocytes. *Infection and Immunity* 50 (2): 5616-571. 1985. 8. Jacobsen, K.L. Unpublished data. 9. Emodi, G., Just, M., Interferon production by lymphocytes in human milk. *Scand J. Immunol* 3(2): 157-160. 1974. 10. Keller, M.A., Kidd, R.M., Bryson, Y.J., Turner, J.L., Carter, J., Lymphokine production by human milk lymphocytes. *Infection and Immunity* 32(2): 532-636. 1981. 11. Schafer TW, Lieberman M, Cohen M, Carne PE: Interferon administered orally: Protection of neonatal mice from lethal virus challenge. *Science* 176: 1326-1327. 1972. 12. Jacobsen KL, Rockwood GA, Abolhassani M, Evans DL, Chitwood SW, and Charamella L. Kinetics of the large-scale production of bovine leukocyte interferon with 3 different virus inducers. *Am J Vet Res* 49(9): 1441-1446, 1988. 13. Chandra, R.K., Nutritional deficiency and susceptibility to infection. *Bulletin of the World Health Organization* 57(2): 167-188. 1979. 14. Ali, H.M., Scott, R., Toms, G.L., The susceptibility of breast-fed and cow's milk formula-fed infant guinea pigs to upper respiratory tract infection with influenza virus. *Brit J Exp Path* 69(4): 563-575, 1988.