Immune Defence Proteins in Human Milk: Differences between preterm and term deliveries

Report on Waikato Medical Research Foundation
Grant 182

AGFH 01489

May 2010 to May 2011
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Lay summary for WMRF annual report

Milk has multiple functions. Besides providing a source of nutrients to the newborn, it also contains a number of proteins that contribute to the defence against infections. The levels of these proteins may have a big influence on a baby’s health and wellbeing. For many of these proteins, it is not known how their abundance in milk varies in a typical human population, or indeed within an individual over time. Nor is it known whether this abundance is altered when a mother gives birth prematurely. These premature infants are particularly vulnerable to infection and the levels of these proteins in the milk from such mothers may not be the same as for full term gestation, since like the infant, the mammary gland itself follows a co-ordinated development leading to milk production, which is timed to coincide with a normal length gestation.

This project aimed to assess the abundance of five such defence-associated proteins in milk from 30 mothers, 10 from each of three groups – full term deliveries, premature deliveries (32-38 weeks) and very premature (less than 32 weeks). Each volunteer provided milk samples at two different times. The range and variability of the abundance of these proteins was assessed within each study group, between the three groups, and between the two samples from each volunteer.

In total, 29 out of the anticipated 30 volunteers were recruited to provide milk at two time points; 2 weeks and 5 weeks after giving birth. Analysis on them is almost complete, and a preliminary statistical analysis has been done. From the preliminary data to date, it was found that the variability within each group is quite high compared to the major milk proteins, and that this variability is independent of the length of gestation. There is also some variation between the two samples taken at two different times from each mother, but this appears to be less than that between mothers. Further work will solidify these preliminary findings. The final data will be prepared for publication in a science journal.
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1. **Selected results presented in more detail**

The five host-defence associated proteins that were measured were Immunoglobulin G, Immunoglobulin A, Secretory Component, Lactoferrin and Complement C3. These were chosen based on their known presence in milk and their known biological roles in the defence against pathogens.

The variability in the IgG levels in the full term gestation group is shown below (Fig. 1) as an example of the typical pattern of variability observed in the study. Similar graphs have been produced for all the samples analysed to date in all three of the groups (“T” full term; “P” premature; and “V” very premature). The large differences between the 2 week and 5 week samples in two of the mothers was quite distinct from the remainder, and may have been due to particular environmental conditions that changes between the two time points. The most likely cause of this is the presence of a pathogen, which is known to cause large differences in the abundance of some host-defence associated proteins. The equivalent data for the other four milk proteins gave essentially similar results for each of the groups.

**Figure 1:** IgG concentrations (in µg/ml of milk) for “T” group volunteers’ two week and five week sample.

One sample gave particularly anomalous results. This was the 5 week sample from one of the mothers in the term group. The levels of IgG (see Fig. 1), C3 and Secretory component (see Fig. 2) were all much higher in this sample compared with any of the other samples. This is most likely due to an inflammatory response due to the presence of pathogens, and indicates mastitis that might progress to overt clinical
symptoms. This information was relayed back to the mother via the hospital staff so that appropriate follow-up could be initiated.

Figure 2: Secretory Component concentrations (in µg/ml of milk) for “T” group volunteers’ two week and five week sample.

2. Future work to complete the project

Recruitment of volunteers to donate samples took longer than anticipated, and significantly overlapped with the time period allocated to analysis of the samples, which was done by a BSc (Tech) student enrolled at the University of Waikato as an industry work placement, from July 2010 to February 2011. Because of this, analyses of four of the five milk proteins were completed, except for two samples which became available only after the most of the analyses had been done. Development of the assay for the analysis of the fifth protein, lactoferrin, was completed, but quantification of the samples themselves was not able to be completed by the time the studentship expired.

These additional analyses will be completed in the period June to July, 2011, and this will be followed by a comprehensive statistical analysis which will provide estimates for the degree of variability within a volunteer over time, within a group and between
groups, together with a measure of the significance of any differences in these variabilities. This will be completed by August, 2011.

A manuscript will then be prepared for publication of these results in a scientific journal. In addition, the study and its results will be presented at the next Waikato Medical School seminar series. Also, a presentation will be made to a local Iwi group in order to explain the purpose and outcome of the research to them, in fulfilment of the wish expressed by the Waikato DHB Kaumatua Kaunihera.