SPLASH!® milk science update
March 2016 Issue

INTERNATIONAL MILK GENOMICS CONSORTIUM

This month's issue features de-stressing with dairy, more about prolactin's complex role in the human body, and how lactoferrin can be used to treat infections and develop flu vaccines for infants.

De-stressing with Dairy

- **Stress alters the composition of bacterial communities in the gut because stress-associated hormones speed the growth of some pathogenic species.**
- **Through various mechanisms, alterations in the composition of gut bacterial communities also appear to change mammals' stress response.**
- **Research into how gut microbes influence the ability to handle anxiety is very recent. But the research suggests that some key components of milk (and milk products) alter microbial communities in ways that help mammals to overcome feelings of anxiety and stress.**

When, a few years ago, researchers analyzed fecal samples from volunteer undergraduates at Swinburne University of Technology, in Victoria, Australia, they didn't necessarily expect to find evidence of the students' examination stress. Yet the fecal lactic acid levels—reflecting the amount of "good bacteria" of the genus *Lactobacillus* in the students' guts—took a dive during the exam period [1]. In other words, exam stress had caused the volunteers' intestines to become more favorable environments to pathogenic organisms. As the exams went on, things only got worse: the researchers observed day-by-day reductions in the undergraduates' fecal lactic acid levels. This couldn't have been because exam-period diets were messing with the students' health—the only significant dietary change was an increase in coffee consumption.

Since this finding was reported in 2008, the interplay between stress and the composition of bacterial communities in the gut has been probed much further. Not only does stress appear to alter the body's flora, but the reverse also seems to be the case: gut microbiota seem to influence the brain and behavior, in particular altering how mammals respond to stress. The evidence in the latter direction is still somewhat tentative, but nonetheless suggests a cheap and simple anxiety-busting remedy: eating foods that tend to shift gut bacterial populations towards healthier compositions.

The causal chain in the direction that the Swinburne University students demonstrated begins with a rise in the levels of hormones that are induced by stress. In mice, *Escherichia coli* levels have been shown to multiply 10,000 times following a dose of norepinephrine (noradrenaline) [2], a hormone made by cells of the nervous system that readies the brain and body for action, causing a sense of anxiety. Many other bacterial species react in similarly dramatic ways in laboratory tests, including *Salmonella enterica*, a bacterial species sometimes found in gone-off eggs.

The causal chain going the other way centers on the idea that gut microbiota can influence the reactivity of the immune system, with the proteins they produce priming it to respond to stressors in an accentuated manner. This, in turn, implies changes in the way that mammals behave in response to stressors. For example, a major metabolite generated by good gut bacteria is butyrate, which is important in the inflammatory response of the colon. Its action is partly down to how it influences gene expression via an enzyme, which it inhibits, called histone deacetylase inhibitor (HDAC). HDAC regulates transcription. So, when HDAC is bound to a gene's "on-switch" in immune cells called T-cells, the presence of butyrate increases the production of proteins encoded by that gene. This has been observed for the Fas promoter (or, "on switch"), for which the presence of butyrate leads to an increase in the number of Fas receptors on the surface of T cells [3]. That makes them more ready to commit cell suicide—which is thought to reduce gut inflammation.

That's just one example of how gut microbiota can influence physical stress. But there are likely other mechanisms at play, through which populations of bacteria in the gut alter mammals' responses to more psychological forms of stress.

This is demonstrated by a paper published only a few months ago [4], which reports how Andrew Tarr and his colleagues, of Ohio State University, in Columbus, investigated the role of medium-sized sugar molecules—which are found in high levels in breast milk, and well known to shape bacterial communities in infant guts—in altering anxiety-like behaviors. For various reasons, including the ethics of deliberately stressing out human babies, the researchers worked with mice.

Over a two-week period, the mice in the experiment were fed either their usual laboratory diet, or a version of it spiked with one of the most common medium-sized sugars found in human breast milk (some mice were given 3'sialyllactose,

while others received 6'sialyllactose). Then the established cohorts of male mice had anxiety forced upon them through the introduction of an aggressive male intruder, for two hours per day over almost a week. These aggressive intruders were mean: if they didn't initiate an attack or get attacked by a member of the freaked-out established cohort within the first five minutes, the researchers replaced the intruder with a better bully. Typically, the researchers observed the mice in the established cohort cowering, fleeing and exhibiting other kinds of subordinate behavior. The anxiety was also evident in levels of the stress hormone corticosterone, in the animals' blood, in samples taken shortly after the final session in a bully's company.

When the mice were suitably stressed out, the real test came in the form of how they responded to being placed in a new type of environment. Given the option of spending time in the dark part of a box versus in the light, mice that are suffering from feelings of anxiety displayed a preference to cower in the dark. Similarly, such mice prefer closed areas to wide open spaces.

When the researchers placed the mice in these kinds of environments, they found that only mice on the control diet behaved anxiously; those fed either kind of medium-sized sugar were as unreserved in exploring the light or open areas of their new environments as mice fed the control diet and that were never exposed to an aggressive intruder. In short, it appeared as though the sugars had somehow helped the mice "bounce back" to a psychologically normal state following a period of high anxiety.

Why this would happen is not entirely clear. It could be that the sugars have a direct impact on the animals' brains. But a likely avenue is that their effects are mediated through gut microbiota. Tarr and his team probed this [4]. Genetic sequencing of the bacterial communities found in the animals' guts after they were stressed out revealed significant differences between the mice fed the normal laboratory diet and those whose meals were spiked with milk sugars. (Meanwhile there was no statistically significant difference between the microbiota of mice fed 3'sialyllactose and mice fed 6'sialyllactose).

Tarr and his colleagues suggest that these sugars helped in the maintenance of a healthy gut bacterial community during a period of high stress. Their absence in the diet thus allowed hormonal shifts involved with anxiety to alter community structure—rather like the undergraduates suffering through their exams.

So, might dairy help people overcome anxiety in general? The lack of studies in humans makes that difficult to answer. But it does seem to help mice overcome a common source of human anxiety—the abrupt discontinuation of nicotine, which occurs when someone quits smoking. In a recent study by Iranian scientists, Negin Noori et al. [5], kefir—the fermented milk drink—was reported to alleviate nicotine cessation-induced anxiety. In this case, however, the researchers point the finger at the amino acid tryptophan, which is both abundant in kefir and a precursor of serotonin, the neurotransmitter whose low levels are linked to the anxious feelings that those who quit smoking often suffer from.

"Sertraline is a very famous prescription pharmaceutical for the treatment of depression, social anxiety disorder, and also panic disorder," wrote Noori, of the University of Tehran, in an email. "We found that kefir is as capable as sertraline to reduce significantly the signs of anxiety and depression." Alas, she has no plans to conduct human trials, even though those involved would be volunteering to experience anxiety by choosing to give up smoking.

1. Knowles, S. R., et al. (2008) Investigating the role of perceived stress on bacterial flora activity and salivary cortisol secretion: A possible mechanism underlying susceptibility to illness. Biological Psychology 77, 132–137.
2. Gur, T. L., et al. (2015) Stress and the commensal microbiota: importance in parturition and infant neurodevelopment. Frontiers in Psychiatry 6 (5), 1–6.
3. Zimmerman, M. A., et al. (2012) Butyrate suppresses colonic inflammation through HDAC1-dependent Fas upregulation and Fas-mediated apoptosis of T cells. AJP: Gastrointestinal and Liver Physiology 302 (120), G1405–G1415.
4. Tarr, A. J., et al. (2015) The prebiotics 3'Sialyllactose and 6'Sialyllactose diminish stressor-induced anxiety-like behavior and colonic microbiota alterations: Evidence for effects on the gut–brain axis. Brain, Behavior, and Immunity 50, 166–177.
5. Noori, N., et al. (2014) Kefir protective effects against nicotine cessation-induced anxiety and cognition impairments in rats. Advances in Biomedical Research. 3, 251.

Contributed by
Anna Petherick
Professional science writer & editor
www.annapetherick.com

Prolactin: One Hormone, Many Effects

- **During lactation, the hormone prolactin targets the mammary gland and several other tissues, including the intestines and kidneys.**
- **Each target tissue has a receptor specific to prolactin but responds to prolactin's signal with a different cellular action.**

- **New research in pigs identified 24 different mRNA transcripts of the prolactin receptor gene, many of which were unique to particular tissues.**
- **Tissue-specific expression of the prolactin receptor gene may explain tissue-specific responses to prolactin during lactation.**

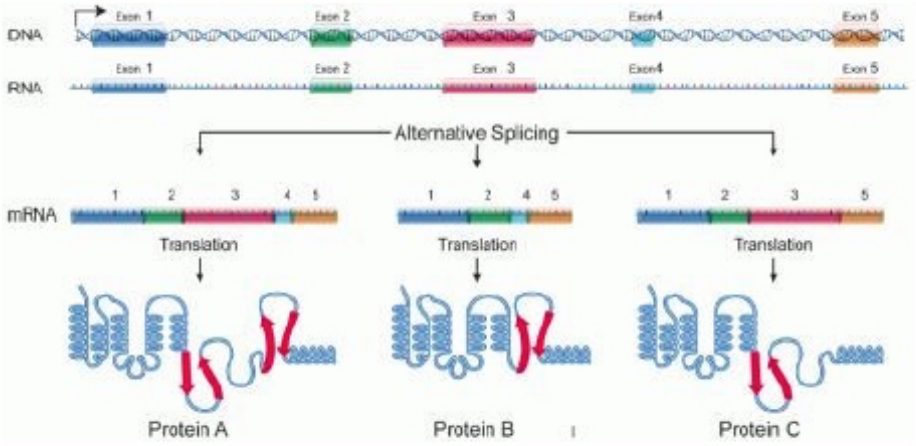
Hormones are the body's bike messengers, carrying important information from the endocrine glands (e.g., thyroid, pituitary, ovaries) to tissues throughout the body. Whereas GPS helps bike messengers find their intended recipients, hormones know they have found their target tissue when they are able to bind to hormonal receptors attached to a cell's surface. Like a key fitting into a lock, hormones only bind to their specific receptor. Turning the key and opening the receptor's lock allows the hormone to deliver its message to the cell, which responds by taking a particular action. The lactation hormone prolactin (PRL) has more than one target tissue, with prolactin receptors (PRLR) found in mammary glands, intestines, kidneys, ovaries, and even the heart [1]. Mammary gland cells respond to PRL's signal by secreting milk and initiating lactation, whereas cells of the intestines respond by increasing their absorption of calcium [2, 3]. How does the same hormonal signal result in the delivery of such different messages?

The results of a new study in pigs suggest that each tissue may have a different response to PRL's signal because each tissue has a unique receptor [1]. Although the mammary glands, intestines, and kidneys have the same DNA recipe to make PRLR (the PRLR gene), each appears to use a different combination of ingredients to get to the finished product (the PRLR protein). Thus, rather than a key that fits only one lock, PRL may better be described as a master key able to fit into many PRLR locks, each lock prompting a different physiological response.

It's all about how you splice it up

The hormone PRL is a chemical messenger produced by the pituitary gland. Traveling through the body's bloodstream, PRL has the potential to interact with every type of tissue in the body, but it can only communicate with tissues that express PRLR on their cell surface. The lock and key analogy for hormones and their receptors is fitting (pun intended!) because it is their specific shapes that allow the two to bind together, resulting in communication between hormone and cell.

For hormones and receptors (and all other proteins), shape is determined by the order of amino acids, which is in turn determined by the DNA instructions of the gene (i.e., the order of the DNA bases A, C, T, and G). Given this, it may seem surprising that a new investigation by Schennink and colleagues discovered that the actual order of the DNA bases for the PRLR protein differed depending on the tissue in which they were expressed [1]. How do cells take identical genetic information and make different final products? It is all about how you "splice" the gene up.



One Gene, Many Proteins

Messenger RNA (mRNA) is the intermediary molecule between DNA code and amino acid. Not all DNA letters in a gene are used to make amino acids; the coding portions of genes are called exons and the non-coding portions are called

introns. Before the mRNA code is translated into a protein, the introns need to get spliced out. During this mRNA processing stage, however, particular exons may also get spliced out. While this sounds like some type of mutation or malfunction, it is actually intentional and quite an amazing biological trick. One gene has the potential of producing multiple proteins depending on which exons are maintained and which are spliced out.

The different mRNA versions of the same DNA code are referred to as splice variants, reflecting the different ways in which the exons were spliced out during mRNA processing. Imagine a gene for protein Z with 12 exons. One splice variant could be made up of exons 1–5, exon 8, and exons 10–12, and another splice variant could be exons 2–10. Both make protein Z, but the proteins differ in their amino acid composition, and thus, in their final structure.

In their examination of PRLR gene expression in pig tissues, Schennink and colleagues [1] found a lot more complexity than simply two splice variants, however. Across the seven tissues they identified 24 different splice variants of the PRLR gene: nine different exons were used as first exons (referred to as exons 1.1–1.9), each of which could be followed by exon 2 and exon 2.1, followed by only one of those exons, or by neither [1].

Trying to make order out of this complexity, Schennink et al. [1] compared splice variant expression by tissue type. Amazingly, certain splice variants were unique to, or expressed in significantly greater amounts, in particular tissues. For example, pig heart tissue expressed the transcript that used exons 1.5, followed by exons 2 and 3 (1.5/2/3) at levels 20 times higher than any other tissue. In contrast, expression of the transcript 1.5/3 was highest in the mammary gland and 1.6/2/3 was enriched only in kidney tissue [1].

From structure to function

Each splice variant identified is derived from the same PRLR gene, and each produces a protein that effectively binds PRL. But because each differs slightly in the exons translated into a protein, each tissue's PRLR differs slightly in their structure. For proteins, structure determines function. This suggests that the tissue-specific splice variants of PRLR potentially result in PRLR with tissue-specific functions. The emphasis here is on suggests, as receptor function was not part of Schennink et al.'s study [1]. But it is certainly not a stretch to take their findings and hypothesize that tissue-specific PRLR structures result in tissue-specific functions of PRL. And what an elegant evolutionary hypothesis it is: the pituitary produces one chemical signal that results in various physiological responses from different tissues simply by altering the order of exons in the targets. Who needs a fleet of bike messengers when you can send just one capable of delivering unique messages to different recipients?

Schennink and colleagues [1] propose that the complexity of PRLR genetic expression supports a role for PRL as the regulator of the coordinated, but diverse, physiological responses of mammary and peripheral tissues during lactation. Successful lactation, after all, requires much more than secreting milk from cells in the mammary glands. Macro- and micronutrients must travel from various locations throughout the body to those cells, and other tissues must adapt to the changing physiological demands of lactation (e.g., increased cardiac output due to greater blood flow to the mammary glands) [1]. Although the specific actions of PRL on many tissues throughout the body are still being elucidated, expression of unique PRLR splice variants in mammary glands, heart, intestines, and kidneys *only during lactation* strongly suggests that PRL is essential to milk production and the physiology of a lactating female.

Homologs in humans

Pigs have 24 splice variants of the PRLR gene. But what about humans? Are we equally as complex, or perhaps even more so? It is not possible to duplicate Schennink et al.'s [1] work with human tissues, but the researchers provide strong evidence to suggest that pigs are appropriate models for the human PRLR gene. Specifically, they identified a homologous (similar in sequence) human PRLR first exon to that identified from pig intestine and kidney [1]. The human exon also was uniquely expressed in these tissues in humans, offering strong support for a conserved function of PRL (calcium homeostasis) in these tissues in multiple mammal species.

Schennink et al. [1] did not get lucky by finding a homologous gene in their pig tissue samples. They selected pigs as their animal model specifically because pigs have several physiological similarities to humans and are more genetically similar to humans than are mice or rats. While understanding the specific actions of PRL in humans is the ideal, their study (and numerous others, like that of Wongdee et al. [2]) demonstrates that modeling PRL function and genetic expression of its receptor in non-human mammals has strong implications for our understanding of PRL during human lactation. Whether in the rat, pig, or woman, PRL is one hard-working hormone.

1. Schenninck A, Trott JF, Manjarin R, Lemay DG, Freking BA, Hovey RC. 2015. Comparative genomics reveals tissue-specific regulation of prolactin receptor gene expression. Journal of Molecular Endocrinology 54: 1–15.
2. Wongdee K, Teerapornpuntakit J, Sripong C, Longkunan A, Chankamngoen W, Keadsai C, Kraidith K, Krishnamra N, Charoenphandhu N. 2016. Intestinal mucosal changes and upregulated calcium transporter and FGF-23 expression during lactation: contribution of lactogenic hormone prolactin. Archives of Biochemistry and Biophysics 590: 109-117.
3. Newmark, L. M. 2016. *Prolactin targets intestines too.* SPLASH! milk science update.http://milkgenomics.org/article/prolactin-targets-intestines-too/.

Contributed by
Dr. Lauren Milligan
Research Associate
Smithsonian Institute

Fighting Infections Using Lactoferrin Capsules

- **Lactoferrin protein in breast milk helps protect infants from infections.**
- **Some of the antimicrobial activity of lactoferrin is due to a small fragment of the protein.**
- **A slight change of the structure of the lactoferrin fragment drives the formation of capsules than can destabilize membranes of bacteria and mammalian cells.**
- **The capsules can be used to transfer molecules into mammalian cells.**
- **The goal of the research is to use the capsules to treat microbial diseases and in gene therapy.**

The ability of lactoferrin to kill infections due to bacteria, fungi, and viruses in the first few months of life has long been recognized as one of the big benefits of breastfeeding for newborns. The antimicrobial prowess of lactoferrin is based on a tiny, six amino acid-long stretch of the protein called lactoferricin [1]. Researchers have long tried to exploit this peptide as an effective antimicrobial agent. Successes have been achieved, but the search for even better strategies continues.

A recent paper in *Chemical Science* [2] has brightened the outlook for the therapeutic use of another lactoferrin peptide—this one a tiny six amino acid-long fragment. By replacing two of the amino acids in the peptide, scientists from the National Physical Laboratory and University College London in the United Kingdom created a structure that contains what the researchers describe as a "self-complementary sequence." The presence of this sequence causes the peptides to associate with one another. The result is the formation of hollow capsules that are uniform in size, ranging between 20 and 200 nm, about the size and shape of those formed by certain types of viruses.

Poking holes in membranes

The capsules have a double-whammy effect. The first effect is the destabilization of the lipid-based membrane that normally functions to keep cells intact and alive. To demonstrate this destabilization, the researchers formed bilayers of lipid in a solution. Lipids that are a part of biological membranes have two portions—one, termed the tail, associates with water (hydrophilic) and another, termed the head, which does not associate with water (hydrophobic). In a water-based solution, the lipids will spontaneously form a two-layered structure in which the tails are exposed to the solution with the heads sandwiched between. In the sandwich analogy, the slices of bread are the lipid tails and the filling constitutes the lipid heads. A compound that can be detected is then introduced in the solution on one side of the bilayer. The solution on the opposite side is free of the compound. Destabilization of the bilayer can then be revealed by the movement of the compound to the other side of the lipid bilayer. In this way, the researchers demonstrated the ability of the capsules to bind to the bilayer and render it leaky. Further sleuthing indicated that the leakiness was due to the formation of pores made of the lactoferrin peptide in the lipid bilayer.

For bacteria, a leaky membrane does not bode well. The researchers tested whether the results in the lipid bilayer experiment extended to real-life bacteria with different types of membranes. They focused on two species of Gram-negative bacteria capable of causing infection (*Escherichia coli* and *Pseudomonas aeruginosa*) and the Gram-positive pathogen *Staphylococcus aureus*. For all three, suspensions of live bacteria were decimated following addition of the lactoferrin peptide capsules.

Like broad-spectrum antibiotics, the capsules killed different types of bacteria. The problem with broad-spectrum antibiotics has been the development of resistance by the bacteria. However, resistance to membrane destabilization may not develop as easily.

Putting genes in cells

The second cell-fighting effect of the capsules relies on their hollow shell structure, which allows the capsules to be exploited to transport molecules inside cells. The target cells can be bacteria or mammalian cells. In the case of bacteria, the aim would be to ferry a toxic molecule inside bacteria. For mammalian cells, the aim is to use the capsules in gene therapy. This therapy can proceed two ways. In one approach, a defective gene in a cell is replaced and the beneficial function associated with the defective gene is restored. In the other approach, a gene associated with an undesirable function is blocked and the molecule it codes for is not manufactured. This binding-followed-by-transport route occurs naturally when certain types of viruses infect cells, which lead the researchers to dub the capsules "synthetic antimicrobial

viruses."

As a proof of the gene therapy concept, the utility of the capsules in blocking genes in cancer cells was explored. In one experiment, the lactoferrin peptide capsules containing a fluorescent probe were exposed to the cancer cells. The cell interiors fluoresced, confirming delivery of the payload inside the cells. Next, the researchers packed the capsules with a type of ribonucleic acid (RNA), termed small interfering RNA (abbreviated siRNA). Many siRNAs exist—each one targets a unique stretch of another RNA species. The binding between the RNA types blocks the manufacture of protein [3]. When the capsules contained various types of siRNAs, production of the messenger RNAs that carry the information for various cancer cell proteins was shut down. Supplying empty capsule alone did not shut down protein manufacture.

The upshot is that the altered lactoferricin capsules can destabilize biological membranes and can ferry molecules inside bacteria and mammalian cells. From tailored destruction of disease-causing microbes to human gene therapy, the lactoferrin peptide capsules have significant potential.

1. Schibil D.J., et al. (1999) The structure of the antimicrobial peptide center of lactoferrin B bound to sodium dodecyl sulfate micelles. FEBS Lett. 446, 213-217.
2. Castelletto, V., et al. (2016) Structurally plastic peptide capsules for synthetic antimicrobial viruses. Chemical Science DOI:10.1039/C5SC03260A.
3. Li L.C. (2008) Small RNA-mediated gene activation. In, RNA and the Regulation of Gene Expression: A Hidden Layer of Complexity. Caister Academic Press. Poole, UK

Contributed By
Dr. Brian Hoyle
Square Rainbow Limited, science wordsmithing
hoyle@square-rainbow.com

A Milk Protein Could Help Produce a Flu Vaccine for Newborns

- **Vaccines often rely on additives called adjuvants to increase their effectiveness.**
- **A commonly-used adjuvant, aluminum hydroxide gel (ALUM), does not effectively boost the immature immune system in preterm infants and newborns.**
- **There is no effective flu vaccine for infants under 6 months of age, which contributes to their high morbidity and mortality.**
- **A new study finds that the milk protein lactoferrin can serve as a safe and effective adjuvant in a neonatal mouse flu vaccine.**
- **Neonatal mice given an influenza vaccine containing bovine lactoferrin adjuvant exhibited an equivalent antibody response and ability to neutralize the influenza virus as mice given the vaccine containing ALUM.**

The flu virus can be deadly, particularly in the very young and the very old. Unfortunately, there is no flu vaccine available for use in infants under 6 months of age, and currently the best way to protect these babies is to vaccinate their parents and anyone else in close contact with them. Developing a safe and effective vaccine for use in very young infants could greatly help reduce their morbidity and mortality [1–3].

A new study, conducted by Michael Sherman at the University of Missouri School of Medicine, finds that a major breast milk protein called lactoferrin, could potentially be used to develop a flu vaccine for use in newborns [4].

Lactoferrin previously has been shown to play a role in some of the beneficial immune effects of human breast milk. "Lactoferrin is a prominent breast milk protein, and limited studies report breastfeeding enhances MMR vaccination at 12 to 15 months," writes Sherman in an email.

Preterm infants and newborns have an immature immune system that not only makes them susceptible to infections, but also reduces the effectiveness of vaccines. Vaccines often use additives called adjuvants to enhance the body's immune response. Unfortunately, one of the most commonly used adjuvants, aluminum hydroxide gel (ALUM), doesn't effectively stimulate the immune system in newborns [5, 6].

The researchers hypothesized that lactoferrin might be able to replace ALUM as an adjuvant when used to immunize neonatal mice against the influenza virus. "We were delighted our hypothesis was correct," Sherman writes. The successful result in mice could potentially lead to the development of a safe neonatal influenza vaccine in humans.

One of the problems in neonates is that certain immune cells known as dendritic cells are still immature, leading to a

reduced immune response. “I have been studying lactoferrin (LF) for 18 years; we have been investigating how LF increases dendritic cell numbers and function in neonates using a mouse model,” Sherman writes. “We had a grant to study whether LF enhanced influenza immunization in these dendritic immature animals,” he writes. “Human infants have this same immaturity in dendritic cells and that is why vaccines fail in early life, along with other immune deficits,” Sherman writes.

When ALUM is injected as part of a vaccine, it recruits immune cells called neutrophils to the injection site, where they secrete lactoferrin. The researchers theorized that by directly injecting lactoferrin as an adjuvant instead of ALUM, they could skip the neutrophil recruitment step and could speed up the recruitment and maturation of dendritic cells.

When the researchers immunized 3-day old mice with a flu vaccine containing either bovine lactoferrin or ALUM, they found that both vaccines led to equivalent antibody production and were able to neutralize the flu virus. Furthermore, both worked considerably better than the vaccine that didn’t contain an adjuvant. This result suggests that lactoferrin could serve as a safe and effective adjuvant for an influenza vaccine in neonatal mice.

In a follow-up experiment, Sherman plans to use the mouse model to study secondary infections, such as pneumonia, caused by the influenza virus and “determine whether influenza pneumonia can be prevented,” he writes. Lactoferrin could potentially be used as an adjuvant in other vaccines, and eventually lead to the development of a human flu vaccine that’s safe for use in newborns.

“A human vaccine containing lactoferrin could potentially be developed within about five years,” Sherman writes. “After we show infection can be prevented in infant mice, we wish to develop a vaccine containing lactoferrin for newborn infants,” he writes. “We are particularly interested in immunizing very preterm infants at hospital discharge,” Sherman writes.

1. C.J. Hebert, C.M. Hall, L.N. Odoms, Lessons learned and applied: what the 20th century vaccine experience can teach us about vaccines in the 21st century, Hum. Vaccin. Immunother. 8 (2012) 560-568.
2. B.R. Kirkwood, S. Gove, S. Rogers, J. Lob-Levyt, P. Arthur, H. Campbell. Potential interventions for the prevention of childhood pneumonia in developing countries: a systematic review, Bull. World Health Organ. 73 (1995) 793-798.
3. Committee on Infectious Diseases, Recommendation for prevention and control of influenza in children, 2015—2016, Pediatrics 136 (2015) 1-17, http://dx.doi.org/10.1542/peds.2015-2920.
4. M.P. Sherman, C.J. Pritzl, C. Xia, M.M. Miller, H. Zaghouani, B. Hahm, Lactoferrin acts as an adjuvant during influenza vaccination of neonatal mice, Biochem. Biophys. Res. Commun. 467 (2015) 766-770http://dx.doi.org/10.1016/j.bbrc.2015.10.067.
5. P. Marrack, A.S. McKee, M.W. Munks, Towards an understanding of the adjuvant action of aluminum, Nat. Rev. Immunol. 9 (2009) 287-293.
6. A. Mori, E. Oleszycka, F.A. Sharp, M. Coleman, Y. Ozasa, M. Singh, D.T. O’Hagan, L. Tajber, O.I. Corrigan, E.A. McNeela, E.C. Lavelle, The vaccine adjuvant alum inhibits IL-12 by promoting PI3 kinase signaling while chitosan does not inhibit IL-12 and enhances Th1 and Th17 responses, Eur. J. Immunol. 42 (2012) 2709-2719.

Contributed by
Dr. Sandeep Ravindran
Freelance Science Writer
Sandeepr.com

Funding provided by California Dairy Research Foundation and the International Milk Genomics Consortium