ANTIBODIES FOR HEALTHY GUT FUNCTION

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TAKE HOME MESSAGE

The gastrointestinal tract is colonized by trillions of microorganisms termed the microbiome. These organisms have pivotal roles in the immunological, metabolic, nutritional, and physiological functions of the host and help keep the body in immune homeostasis, immune balance.

The gastrointestinal tract is at the major intersection of the immune system and microorganisms. Antibodies, immunoglobulins, are large proteins produced by the gut-associated lymphoid tissues (GALT) found in the lining of the intestines.

These antibodies help limit the growth of gut microorganisms, and pathogens that enter via the oral route. When the specificity or levels of immunoglobulins produced by the intestines is in insufficient quantities to protect the young, administration of commercially-available antibodies may be warranted.

MICROBIOME

The gastrointestinal tract (Bauer et al., 2006) and skin (Oh et al., 2014) of animals are colonized by trillions of dynamic ecosystems of microorganisms (Bauer et al., 2006). In totality it is estimated that these organisms express 10-fold more genes than does their host's genome. (Ley et al., 2006; Belkaid and Hand, 2014). As a community, the microorganisms in and on the body are termed the microbiome, and play key roles in the immunological, metabolic, nutritional, and physiological functions of the host (Bäckhed et al., 2005; Wu and Wu, 2012; Scott et al., 2013; Ji and Nielsen, 2015).

The regulatory functions of the microbiome are so vital that the microbiome is considered by some as a separate organ (O'Hara and Shanahan, 2006; Baquero and Nombela, 2012; Ji and Nielsen, 2015).

HOMEOSTASIS OF IMMUNE AND INTESTINAL FUNCTIONS

The intestine is constantly exposed to fluids, foodstuffs, microorganisms, and inorganic (Hooper et al., 2002) and organic materials. Among its many functions are: obtain nutrients, protect the host from infection, and modulate immunological homeostasis of the host (Hooper and Macpherson, 2010; Hooper et al., 2012; Wu and Wu, 2012; Brown et al., 2013; Levesque, 2014).

The lining of the intestine is made up of a single layer of epithelial cells which is highly permeable to permit fluids and nutrients to be transferred to the blood. The gastrointestinal tract has a complex system of immune cells immediately below the epithelium (Tomasello and Bedoui, 2013) that produces antibodies that inhibit the attachment of pathogens to the gut wall and help keep the intestines in microbial and immune homeostasis.

The importance of the gastrointestinal system for defense against orally-acquired infections is supported by the presence of high concentrations of immune cells in the intestines. About 70% of the immune system is represented within the gut-associated lymphoid tissues (GALT) (Jung et al., 2010; Vighi et al., 2010) with 80% of the body's immunoglobulin-producing plasma cells (Vighi et al., 2010) Peyer's patches, residing here as well.

As part of the digestive system's protective mechanisms, when the gut is exposed to antigenic pathogens, the plasma cells will produce immunoglobulins, antibodies, specific to the organisms that stimulated the response. Immunoglobulins, antibodies, are large Y-shaped proteins that bind to the antigens that initially triggered their production. Immunoglobulins attach to and "mark" the pathogen for destruction by immune factors and cells. Antibodies also inhibit the binding of microbes to the intestinal walls, decreasing the likelihood of infection.

There is constant interaction between the members of the gastrointestinal microbiome, immune networks (Shirkey, et al., 2006; Nicholson et al., 2012; Ji and Nielsen, 2015), and the intestinal epithelium cells (Shulzhenko, et al., 2011; de Vrese and Schrezenmeir, 2007; Goto and Ivanov, 2013). Exposure to gut microflora is essential for the full development of the immune structures in the intestines (Tomasello and Bedoui, 2013), and the microbes also have major influences on the structure and function of Peyer's patches (Barman et al.,1997) and other immune components of the gut.

Significant bi-directional influences between the microbiota and the immune system (Tomasello and Bedoui, 2013) play pivotal roles in defending the body from infection and mutating cells, and maintaining the host's immune and physiological homeostasis (Mason et al., 2008; Wu and Wu, 2012; Goto and Ivanov, 2013; Belkaid and Hand, 2014).

Imbalances in concentrations and composition (Belkaid and Hand, 2014) of microbiota affect immunity and local and systemic inflammation in areas distal from the intestines (Ichinohe, et al., 2011; Hand et al., 2012; Belkaid and Naik, 2013; Iida et al., 2013; Belkaid and Hand, 2014). As an example, broad-spectrum oral antibiotic treatment results in changes of the gut flora and influences immunological responsiveness to infection with influenza (Ichinohe et al., 2011).

IMMUNE PROTECTION IN THE NEWBORN

Born agammaglobulinemic, without significant amounts of protective antibodies (Hurley and Theil, 2011), newly born mammals, such as pigs and cows, are vulnerable to infection (Bauer et al., 2006) and other environmental challenges until their own immune systems and microbiome mature.

Initial protection from infection is through the first milk, colostrum, which is passively transferred from the mother (Bauer et al., 2006; Langer, 2009) for the first few days after birth. As the newborn's immune system matures and continues to nurse, the concentration of immuno-globulins and proteins provided in mother's milk decreases (Langer, 2009).

Ingestion of colostrum by the young significantly affects development of the gastrointestinal tract (Blum, 2006) and its microbiome (Cabrera-Rubio, et al., 2012). In pigs and cows, for the first 12-24 hours of life, whole antibodies move easily from the intestine into the circulatory system without being digested by enzymes. However within 24-36 hours after birth, "closure" of the digestive system occurs, and intestinal cells become selective in what passes through the gut wall (Staley and Bush, 1985; Blum, 2006; Hurley and Theil, 2011).

Until the newborn's immune and microbiota has developed sufficiently for it to protect itself, the newly born must have adequate amounts of protective antibodies to meet challenges from invading pathogens. If the antigenic challenge is too overwhelming, or the antibody titers are not sufficient or the specificity of the antibodies not appropriate for the challenge, there may not be enough passively acquired antibody to adequately protect the newborn. Insufficient amounts of antibodies require that the young be cross-fostered onto another mother, or given supplemental antibodies (ThePigSite.com).

HETEROLOGOUS TRANSFER OF PASSIVE IMMUNITY

Typically, immunoglobulin-rich colostrum, hyperimmune milk, or hyperimmune eggs are used in humans and livestock to help the newborn defend itself from pathogenic challenges until its own immune and microbiota systems are mature.
A useful extension of our knowledge of passive transfer of protective immunoglobulins is the opportunity to use immunoglobulins from one host for prevention or treatment of disease in a secondary host.

Decades ago, clinicians orally transferred anti-rotavirus human immunoglobulin to three children with primary immunodeficiency syndromes that were suffering from chronic excretion of gastrointestinal rotavirus. The antibody survived passage through the gut and retained its ability to attach to rotavirus. Antigen-antibody complexes were found in the stool for 4-6d post administration of a single dose. For several days after treatment no viral antigen was detected in stools (Losonsky, et al., 1985).

Significant immune protection can also be transferred orally by using antibodies from one species for another (Hammarström et al., 1994; Kovacs-Nolan and Mine, 2004; Hurley and Theil, 2011). Immunoglobulins are fairly resistant to digestion and maintain their immunological activity (Losonsky, et al., 1985; Vega, et al., 2011). Even when immunoglobulins are enzymatically digested, the Fab2 and Fab fragments are still able to bind to the antigen and continue to have neutralizing properties. (Akita and Nakai, 1998; Carlander, 2002).

Hyperimmune products provide a high level of targeted protection to animals. Such proteins are typically produced by stimulating chickens or mammals multiple times with inactivated bacteria and/or viruses. In response to this massive antigenic exposure, the host produces both specific and non-specific immunoglobulins, along with high concentrations of other biological and immune factors that help maintain host immune homeostasis.

Hyperimmune proteins have been studied extensively in humans and other animals and shown to enhance functioning of joints, digestive and other organ systems (Heckert, et al., 1999; Mine and Kovacs-Nolan, 2002; Larsson and Carlander, 2003; Kelly GS, 2003/4; Schade R, et al., 2005; Hurley and Theil, 2011; Xu et al., 2011; Kramski et al., 2012).

EGG PROTEINS AS A SOURCE OF IMMUNOGLOBULINS AND OTHER IMMUNE FACTORS

The hen passively transfers immunity, nutrients, and growth factors to her chicks by means of the egg.

Eggs have been used since antiquity to enhance animal and human health. One of the oldest works on the medical treatment of donkeys, mules, and horses P. Vegeti Renati Digestorum Artis Mulomedicinae libri (Fischer, 2011) mentions egg as a remedy for diseases.

In human applications, the Roman army traveled extensively and had purpose-built hospitals with physicians trained and influenced by the Greeks. Chronicles suggest that raw eggs from the local areas were used to prevent and treat diseases such as dysentery (Lommatzsch, 1903) as the Roman army moved from territory to territory.

In response to antigenic stimulation of hens, either via environmental exposure to pathogens, or injection of microorganisms, antibodies are concentrated in the yolk, along with a wide-spectrum of bioactive elements which are found both in the white and in the yolk of the egg.

Hyperimmune egg is not only rich in immunoglobulins and nutrients, but it contains a multiplicity of pro- and –anti-inflammatory molecules, interferons, chemokines, anti-viral and anti-bacterial biological factors (Wu et al., 2010). Investigators have calculated that there is a 30x greater concentration of small immune factors in a single egg from a “hyperimmunized” hen as compared to a regular egg.

The discovery that immunoglobulin was detectable in egg yolks of vaccinated hens, as well as in the blood, pre-dates the use of an antibiotics (Klemperer , 1893). Although functionally equivalent to mammalian IgG (He et al., 2014), the type of immunoglobulin produced by birds was deemed different enough from mammalian antibodies and was thus designated as “IgY” (Leslie and Clem, 1969).

IgY not found in mammals, but is the dominant class of immunoglobulin found in avian egg yolk. It has multiple advantages over mammalian antibodies. For example, mammalian-derived antibodies can trigger inflammatory processes when they interact with rheumatoid factor or complement. IgY does not interact with either of these immunological factors (Gottstein and Hemmeler,1985; Schade et al.,1991).

Another important advantage of IgY is that for microorganisms, its avidity, i.e., the binding capacity of IgY, is much tighter than the avidity of mammalian antibodies. Additionally, since fats and proteins in egg help protect IgY from enzymatic degradation, chicken immunoglobulins are less susceptible to digestion than bovine antibodies. Also a higher degree of protection is

obtained with egg antibodies as compared with the same amount of mammalian immunoglobulins (Ikemori, et. al. 1997).

Despite the fact that chicken egg is an impressive source of antibodies (Tini et al., 2002), egg-derived immunoglobulins are under- utilized for animal and human applications (Kovacs-Nolan J, Mine Y 2004; Carlander et al., 2002; Schade R, et al., 2005; Xu et al., 2011).

Oral administration of egg antibodies may be a natural means to reduce or eliminate the use of antibiotics and control infections from bacteria and viruses (Kollberg et. al. 2003; Larsson and Carlander, 2003; Kovacs-Nolan and Mine, 2004).

A partial list of pathogens against which IgY antibodies have been produced are: *Helicobacter pylori* (Shin et al., 2002; Wang et al., 2014; Yang et al., 2012), influenza (Yang et al., 2014), *Pseudomonas aeruginosa* infections in cystic fibrosis patients (Kollberg et al., 2003), hemorrhagic and bovine and human rotavirus viruses (Yolken et al., 1988; Kuroki et al., 1997; Sarker et al., 2001; Kovacs-Nolan and Mine, 2004; Li et al., 2014),), canine parvovirus-2 (Van Nguyen et al., 2006), infectious bursal disease (Yousif et al., 2006), *Toxoplasma* (Ferreira Júnior et al., 2012) and *Trypanosoma* (Sampaio et al., 2014).

Egg-derived IgY have also been studied as a less arduous means of producing antibodies against bovine and human diarrheas (Yokoyama et al., 1993; Erhard et al., 1993; Ikemori et al., 1997; Kweon et al., 2000; Vega et al., 2011; Diraviyam et al., 2014), *H. pylori*-induced gastritis (Shin et al., 2002), *Escherichia* coli (Akita and Nakai, 1998), and botulism neurotoxins (You et al., 2014), and snake (Aguilaret al., 2014), spider and scorpion venoms (Schade et al., 2005).

Orally-administered IgY is transferred and absorbed into the circulatory system of a piglet as efficiently as are the IgG antibodies from the sow's colostrum (Yokoyama et al., 1993). Immunoglobulins remain detectable in the neonatal circulation for 24-48h. Additionally, as seen above, specifically-induced egg yolk antibodies are protective against diarrhea in pigs (Yokoyama et al., 1993; Diraviyam et al., 2014) and other mammals.

As a side-benefit, administration of hyperimmune egg has been reported to contribute significantly in daily increases in weight gain (Heckert et al., 1999; Ikemori et al., 1997). Weight gains may be the result of the consumed immunoglobulins helping to control bioburdens in the lumen of the gut requiring less expenditure of energy to maintain gut homeostatic balance.

CONCLUSION

It is becoming increasingly evident that the gastrointestinal system is not merely an organ for digestion of feedstuffs, but has a myriad of other functions. Antibodies produced by the gut-associated lymphoid tissues, and immunoglobulins introduced exogenously, serve to protect the host from pathogens and partner with the gut microbiota and the immune system to achieve homeostatic balance.

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