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The Development of Fetal Immunity from in Utero to Shortly After Birth and Complications that Can Arise from Improper Functioning

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The Development of Fetal Immunity from in Utero to Shortly After Birth and Complications that Can Arise from Improper Functioning

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Abstract

The development of the immune system in humans is highly complex and requires precise timing which is still debated today. Research has shown IL-10 and T-regulatory cells play a significant role in the proper development of the immune system from in utero continuing into months after birth. These components and various cytokines continue to be guiding factors supplemented by the mother via placenta and breastfeeding. The dysregulation of immune development can have detrimental effects on the infant and may lead to autoimmune disorders. In this paper, we aim to further understand the development of the immune system from fetal to early age and discuss factors that can negatively impact the processes.

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General Immune System Purpose

The purpose of the immune system is to provide lifelong protection to things that threaten the organism. Humans undergo extensive development of the immune system in order to have a robust, effective defense system against pathogens without causing significant harm to the human. Humans develop two distinct segments of the immune system which work as a team. There are very complex and intricately timed processes that must work harmoniously for proper immune development. If there are complications within the development there can be serious ailments and disorders that arise in the organism.

Outline of Innate vs. Adaptive Immunity

Humans develop two types of immunity that function as a cohesive unit to provide protection at different times during infections. The first to develop is the innate immunity that is made up of various cell types derived from stem cells and bone marrow (1). This reaction is non-specific and will react the fastest when infectious particles breach the body. Complement makes up a major portion of innate immunity and refers to a series of about 20+ glycoproteins that aid both innate and adaptive cells in clearing pathogens. The innate system also consists of a variety of cells: neutrophils, mast cells, basophils, dendritic cells, eosinophils, monocytes, macrophages and natural killer cells (2). These cells are all programmed to have specific targeting and killing mechanisms or ways to recruit other cells with killing ability. Cells of the innate system recognize and bind to pathogen associated molecular proteins (PAMPs) via their encoded pattern recognition receptors (PRRs). This highly specific interaction launches activation of signaling networks to trigger distinct pathways (3). Cells involved in the innate immune system works in a highly organized manner to destroy the target while educating the

adaptive immune system of the threats that are present, so they are able to react faster and more efficiently the next time the pathogen invades. The downstream effects of the innate system response will increase anti-microbial and cytokine production to instruct the adaptive immune system on how to proceed.

The complement system is a series of plasma proteins, that react to induce a cascade of downstream events leading to inflammatory responses, opsonization, and direct destruction of pathogens. It can be activated via 3 different mechanisms and all will initiate C3 (2). The first is called classical which involves an antibody binding to the pathogen which allows protein C1 to become activated by binding to this complex. This causes a cascade of events ultimately leading to the recruitment of other immune cells, binding to the pathogen to tag for destruction, and the binding of the small fragments directly on the pathogen creating a pore and lysing open the microbe. The alternative pathway begins with C3 and will bind with other factors triggering the same downstream effects with the same results as the classical. Lastly, the lectin pathway is thought to play specific role in fetal immune development (4,5). It begins with the protein lectin binding to specific carbohydrates triggering proteases to becoming active and inducing a cascade of downstream events leading to the production of C5 convertase. This protein is responsible for the continuation of complement protein (C5) cleavage necessary for the pore or, membrane attack complex to form on pathogen surface as well as the continued recruitment of inflammatory modulators (6). This process works very closely with adaptive immunity and links the two immune properties together to work in harmony.

There are various components that aid in connecting the innate and the adaptive immune system together. One of the functions necessary is a proper inflammatory response

which can be triggered in various pathways and cell components including cytokines and interferons. Cytokines are small, soluble molecules that are secreted from nearly all immune cells. They have many functions including dictating cell activation, proliferation and hold the capability of attracting various types of immune cells to the area of pathogen infiltration to help guide the defenses (2). Interferons are a class of cytokines that are secreted by immune cells in response to cell proliferation, viral replication and can help guide anti-inflammatory as well as pro-inflammatory responses (7). Without these proper immune components, there would be no communication between cells and therefore inhibit interactions of innate and adaptive.

The humoral immune responses will become engaged when the innate immune components encounter pathogens and will assist in clearing the pathogen when innate responses are unable to do so by itself. This is crucial in a well-functioning immune system and continues to play a major role in life-long immunity as it has the capacity for immunological memory (2). This is accomplished by the creation of antibodies which are tailored to the specific pathogen that has invaded the body. Some antibodies made will remain in circulation for a lifetime ready to attack faster and more effectively at a subsequent exposure (2). The humoral system is reliant on activated T lymphocytes (T-cells) B lymphocytes (B-cells).

B cells release antigen-specific antibodies which circulate throughout the body via the bloodstream. These antibodies are small proteins that have specialized binding capabilities which trigger downstream effects ultimately leading to the activation of immune cells (8). By binding to the pathogen, this prevents it from attaching to the host's cell which is required for its replication and therefore infection. The binding of the antibody also labels the pathogen for

degradation by other immune cells making antibodies a critical component of both entities of immune functioning.

The 3 types of T-cells are cytotoxic T cell (CD8+), helper T cells (CD4+) and T-regulatory cells (T-reg). CD4+ and CD8+ cells correspond to the glycoproteins they have on the membranes of the cells. When the specific interaction occurs on these receptors, CD8+ cells have the capability of killing pathogenic cells directly by releasing toxic chemicals leading to apoptosis in the target cells (1). CD4+ cells are called helper T-cells because they do not directly kill the pathogen but induce a cascade of signaling molecules to initiate an attack via other immune cells (1). T-regs are very important in preventing overreaction of immune responses and therefore play a unique role in fetal immune development (9). They have the ability to reduce T cell killing activity and prevent the attack of the body's healthy tissues which establishes the body's primary defense mechanism of preventing autoimmune disorders and cancers.

Innate Immunity is present and functional at the time of birth to keep threats contained while the adaptive continues in development throughout the lifetime of the human. It is also exposed first in order to educate the adaptive immune system of what the pathogen is so it can begin to create immunological memory. Therefore, this means the adaptive system has a much slower defense compared to the innate though it offers a more robust and effective killing strategy after the initial exposure (10).

Development of the Immune System in Utero

The development of immunity in utero is a balancing act of preparing to respond to pathogens that will encountered later and preventing an immune response to the "foreign

entity," the mother. The mother's immune system demonstrates a complex idea of immune tolerance during pregnancy which is crucial to avoid mounting a response to the fetus resulting in a rejection. It has been shown that IL-10 and T-regulatory cells (T-regs) play their own unique roles in the mother's immune tolerance during pregnancy. IL-10 is released via CD4+ T cells and monocytes, it is known to be an important immune regulator (2). This can be regulated by inhibiting TH1 cytotoxic responses and suppressing inflammation. Increased levels of IL-10 seen throughout gestation (11) suggests that it could be an essential aspect for creating an immune tolerant environment within the mother. Maternal immune tolerance is necessary for a successful implantation and continued pregnancy. T-regulatory cells have also been shown to play a role in a tolerant environment necessary for proper fetal development (12). They are capable of suppressing various pro-inflammatory mediators to avoid immune activations and rejection of the fetus (12,13,14). This immune tolerance must be maintained during gestation while fetal immune components are developing.

The cells of the immune system continue to develop throughout gestation. B-lymphocytes and T-lymphocytes develop from common lymphoid progenitor cells within the liver and then the bone marrow (2). B-cells will remain in the bone marrow and mature while T-cells travel to the thymus to continue further development and discern into subsets of functional T-cells (2). Both B- cells and T-cells develop around 12-14 weeks gestation while their corresponding receptors are fully diversified by week 26 in utero (15). Although these cells are functional by the third or fourth month of pregnancy, the sterile environment the womb provides does not require the fetal immune system to fend off potential pathogens therefore,

creating a buffer protecting both mother and fetus. This environment is important in keeping the fetus away from harmful pathogens.

Fetal Development and Maternal Immune Protection

The first line of immune defense is passed down from the mother via passive immunity. Immune components such as antibodies have the ability to be passed down through the placenta. These antibodies only last for the first few months as levels begin to rapidly decline after birth yet play a significant role in early life immunity (16). The immune support provided by the mother facilitates broad protective capabilities while the fetus continues to develop the immune system.

Due to the adaptive immune functioning being very limited for the first few months of life, fetal immunity relies heavily on innate immune responses. This is accomplished through various mechanisms of activating innate cell responses and the complement cascade (17,18,20). IgG and IgM are passed from the mother to the baby through the placenta as well as in breast milk (18,19). These antibodies have capabilities of neutralizing toxins, preventing adhesion of pathogens and activating anti-microbial pathways such as complement. Fetal IgM production increases substantially following parturition, (15,16) it can be hypothesized that these initiate complement activation shortly after birth. Complement is subtlety stimulated directly at birth to compensate for the transition to a highly infectious environment from the sterile environment in utero. The complement system is activated in various ways, many of which are supplemented via breast milk (19,20,47). The levels of these maternal immunoglobulins decrease in concentration within the first 6 months of life (15). While these

are decreasing, the young child is building its own immunoglobulin concentrations in hopes to provide a sufficient immune response to possible threats.

Human milk also carries sufficient amounts of immunoglobulins that facilitate passive protection and provide the child with anti-inflammatory factors. One of these factors passed is Interleukin-10(IL-10) both in utero via the placenta as well as in breast milk (21,22). IL-10 is an important immunosuppressive, anti-inflammatory cytokine which aids in balancing an effective immune response to clear a pathogen without causing significant damage to the human. This plays a major role in facilitating immune responses, especially promptly after birth as it has been shown to spike at this time (23). IL-10 is seen in detectable levels throughout the gestational period and in breast milk. Those with IL-10 deficiencies are at an increased risk of developing inflammatory disorders (24,25), supporting the case that IL-10 levels play an important role in immune regulations.

Protective Components in Breast Milk that Guide Future Immunity

The infant's immune system is also supplemented during early life via antibodies passed down via breast milk. This is supported by the make-up of colostrum, the first form of breast milk which has less of a role with dietary nutrition enhancement but acts as a large dose of immune supplements (18). Colostrum is high in vitamins, minerals, antioxidants and antibodies to support the newborn's immune immediately following delivery until the first few days of life (26). These components remain preset in breast milk in lower concentrations throughout the period of breast feeding.

There are various components besides antibodies within breast milk that support the child's immune system throughout the time of breast feeding. Some of the cell types found in

breast milk include: leukocytes, macrophages, neutrophils, natural killer cells (20-24). Various other elements include hormones, enzymes and chemokines/cytokines in which levels fluctuate throughout the feeding period and vary from mother to mother (26,27). The diverse components and concentrations of immune substances help educate and guide the neonatal immune cells to respond properly to pathogens they are most likely to encounter. These cells are capable providing a well-established defense system of immunity before the baby's immune system matures and has the capacity to kill off pathogens.

The components in breast milk also help form the newborns microbiota. The elements within breastmilk guide the development of the immune system and instruct how to deal with potential infectious microbes. These components include oligosaccharides, bifidus and other enzymes which have their own unique role in preventing infections in infants (27,28). Human milk oligosaccharides contribute to the development of the infant's microflora and immune system. By acting via various mechanisms, they protect against many different types of possible infections (29). They have been shown to have anti-bacterial, anti-viral and anti-inflammatory effects. Bifidus refers to a compound present in breast milk *Lactobacillus bifidus.* This bacterium is shown to inhibit pathogenic bacteria such as *S. aureus, Salmonella* and *E. coli* which infants can be highly susceptible to infections from (30). This particular bacterium supports the infant's microbiota formation by actively lowering the pH of the intestines to inhibit growth of harmful pathogens while maintaining an environment for commensal bacteria to reside (30). A newborns microbiota is established early on in life and continues to play a large role in disease prevention.

IgA plays a crucial role in infant immunity as it coats mucosal surfaces to prevent adhesion of pathogens like bacteria and viruses. IgA is the most common antibody found within breast milk. The IgA levels will spike the highest in the first month after birth within the colostrum and will gradually decrease and remain at a lower, stable level for the first year (36). Research has shown that IgA binding can activate the lectin pathway of the complement system (21), proving that IgA plays an important role in active immune defenses at an early age. Presence of IgA at the mucosal surfaces limits pathogen adhesion at common routes of entry like the mouth and nose protecting the infant at a vulnerable time.

There are also enzymes present in breast milk which function to help aid in digestion as well as coordinate immune responses. Enzymes present in breast milk include lysozyme and lactoferrin to maintain an environment that is low in infectious agents (15). These specific components have their unique mechanisms to keep bacteria numbers low so that the antibodies passed via breast milk have the opportunity bind to and limit invasion of bacteria and viruses within the infant.

There are many components within breast milk that are designed to help the infant within the first few months of life whether it is nutritional or to support immune development. This is a critical component in facilitating a functional immune system that is give life-long advantages for survival.

Immune Responses at Birth

Research has shown that there are immediate immune responses after birth. After birth, there is an increase of plasma proteins signifying that there is an immune response which may be crucial for further immune development postnatally. The complement system is

activated within 24 hours after birth (32). This is measured by higher levels of IgM and higher recruitment of proteins C1-C9 which can induce the destruction of pathogens directly (33,34). Although the reason for this increase is unknown, it can be hypothesized as the main protective component when the infant is exposed to the new environment outside of the womb due to the lack of adaptive responses. An increase in complement cascade would allow increased lysis and opsonization of infectious agents as well as activating low amounts of inflammation. In infants, there are lower antibody concentrations compared to those of adults, the increase in complement system is primarily activated via the alternative and lectin-binding pathway which do not require antibodies (21,34). Although there is an overall increase in concentration of complement proteins within the first hours of life, the response is highly controlled with regulatory immune cells to prevent an overreaction and potential harm to the infant. Though this is highly regulated via breast-feeding, infants who are not breast fed will develop their own defense mechanisms as seen in mothers who are unable to provide nourishment in this manner.

The transitions the immune system undergoes from little microbial exposure within the uterus to an environment with many threats of infectious agents is highly elaborate. At birth, there is a significant downregulation of pro-inflammatory cytokines, such as IL-6 and IL-12 (11,31). Research shows there is an increase to regulatory cytokines like IL-10 secreted by B cells at birth without the initiation of an immune response to a pathogen (16). These results indicate the importance of proper immune functioning promptly after birth to prevent an overreaction to the exposure of new pathogens and therefore harm to the infant. Breastfed infants are obtaining key components to building a healthy microbiome including a dose of

"healthy bacteria" (27,35). As the infant's immune system is still naive to pathogens, these anti-inflammatory properties play a key role in preventing the infant's immune system from mounting a response to these commensal bacteria required for a healthy gut (16,23). Significant amounts of pro-inflammatory cytokines, although important later on pose a threat to the infants' health. Pro-inflammatory cytokines such as TNF- α, released by various innate cells, are low at birth and continue to increase during the first 9 months of the infant's life (16). Together, these findings suggest there is active immunosuppression that occurs to protect against excessive inflammation throughout the first few weeks of life. This is accomplished by many different mechanisms and continues to adapt as the infant's immune system matures and exposure to pathogens increases.

There is a balance to children and their exposure to pathogens. The more the child is exposed to at an early age, the more time the immune system has for preparing defensive mechanisms against pathogens. However, overexposure to harmful pathogens could be detrimental in young children.

Continued Development of Immunity Following Birth

As passive immunity begins to diminish, children naturally become more vulnerable to infections. However, as passive immunity weakens, the young child is met with maturing innate and adaptive immunity to continue to build resistance to infections throughout their lifetime (37). Due to the nature of the innate immune system developing first and reacting fastest compared to the adaptive responses, it can be confirmed that the innate immune system is the primary source of protecting the infant (37). The transformation of immune components will occur naturally though can be supported via nutrients that breast milk can provide.

The continued maturation of adaptive immunity takes place making significant strides in the first few months of life. At birth, though the infants will have B and T cells present, they have limited capacity to respond to threats as they are still naïve to most pathogens and therefore express very low levels of co-receptors (38). This not only impedes on B cells immune function it also significantly reduces T cells ability to coordinate immune responses. During the first few months of life, as infants are exposed to more threats in the surrounding environment, B and T cells will mature and increase in functionality. B-cells will begin producing plasma cells that will travel to the bone marrow and aid in long-term immunity by producing antibodies (38). Antibodies are what continue in circulation for long-term immunity. The naïve T-cells and abundance of T-reg cells present at birth continue to decline throughout the first few months. These are replaced by an increase of memory T cells (38). As the individual continues to develop, there are many factors that influence how the immune system develops including genetic variability, environment and methodology of immune responses. This explains how every human reacts to their environment differently and evolution will continue to guide the functionality of immunity to its highest capacity for the remainder of time.

Premature Infants and Improper Development of Immune System

Knowing the highly sophisticated, in-depth processes required for immune development and maturation, it begs the question of how preterm infants are affected. There are many steps that must occur before the child has the capabilities of fending off disease-causing agents; many of which finish development in the last few months of gestation. This includes continued exposure to immune modulators such as anti-inflammatory cytokines, antibodies via the

placenta and maturation of innate immune cells. Infants who are born prematurely face serious medical risks due to the inability to have developed adequate immune responses.

There is no antigen presentation occurring in utero essentially creating a sterile environment protecting the naive immune system until birth. The fetal innate immune responses undergo significant maturation processes in the last 3 months of in utero. (39) If parturition takes place before this as fully occurred, the innate cells are unable to respond to threats sufficiently. Research has shown defective innate immune receptors and therefore dampened responses against bacteria which was recorded by low leukocytes (leukopenia) in preterm infants (39). Leukopenia in newborns can be deadly as they are mostly reliant on innate immune responses due to their adaptive immune responses being immature. Preterm infants have immature monocytes and macrophages which will diminish capabilities of cytokine production and innate responses including complement (39). IFN-γ plays a significant role in the maturation of innate immunity maturation processes due to its function activating macrophages, neutrophils and natural killer cells. Premature infants are known to have low levels of this immune modulator which can have detrimental effects to premature infant's immune responses (39, 40). Sufficient IFN- γ levels are required for proper immune development therefore if this continues to lack, the child will not fully develop a mature immune system. Without proper immune modulators, this leaves little response mechanisms intact and the infant is vulnerable to infectious organisms as well as puts them at a significantly higher risk of having immature immune reactions.

Premature infants lack in the initial upregulation pro-inflammatory responses immediately after birth that is seen in those carried to term. This slight increase in pro-

inflammatory responses aid in preventing infections from the broad exposure of pathogens at birth (39). This correlates to the increased susceptibility to infections that premature infants have with early exposure to pathogens. The lack of pro-inflammatory cytokines increases the risk of serious infections later on, including sepsis (39). Sepsis is defined as an overwhelming inflammatory response throughout the body that can result in the failure of multiple organ systems and even death. The risk of sepsis increases in preterm infants for many reasons, one being the developing fetus does not receive adequate antibodies from the mother and due to their immune systems being immature at the time of birth and its inability to coordinate the highly intricate immune suppression and stimulation of processes necessary for survival. Another reason premature infants are at a higher risk of developing sepsis is due to the microbiota not having sufficient time to fully develop. As previously discussed, the microbiota is highly important in preventing infectious diseases from occurring within infants. During the last few weeks of gestation there are many transitions that occur to the fetal intestines and establishment of the microbiota (24). If this has not had time to occur before the presentation of harmful pathogens, there is no protective barrier within the intestines to outcompete the harmful microorganisms. Many premature infants also remain in a hospital setting for a prolonged period of time which has higher levels of disease and dangerous microbes that can be easily transmitted to the infant if proper care to prevent disease is not taken. Infections and disease within infants can generate immune dysregulation and lifelong disorders of the child.

Theories of Developing Autoimmune Disorders via Dysregulation of the Immune System

There are many theories to explain early life immune impairments and pathologies that develop later in life. Research has shown disorders involving the immune system is dependent

on many different aspects such as genetics, environment and infections at an early age. There are many different immune disorders involving various components which can arise at any point in one's life however, are most commonly encountered in childhood.

Research suggests without proper immune functioning, there is high likelihood in developing conditions with over reactive immune responses such as inflammatory bowel disease (24) and asthma (41). Asthma is chronic inflammatory disorder of the respiratory tract which restricts airflow and causes broncho-constriction most commonly seen in childhood. This is caused primarily due to improper infiltration of immune cells such as T cell, mast cells and macrophages to the respiratory tract and a release of inflammatory cytokines such as histamine signaling to other immune cells resulting in an overwhelming inflammatory response (40). Although the cause of asthma is not fully known, incidence of asthma is seen to be dependent on location suggesting environment plays a significant role in the development of these conditions. Studies have shown that mothers living in rural areas release certain cytokines within breast milk to modulate immune responses to prevent allergies in children compared to breast feeding mothers in urban areas (21). This suggests that the women who are more exposed to allergens and pollens pass on more immune modulators that are advantageous in preventing immune dysregulations. As we have seen prior, IL-10 plays a critical role in preventing significant inflammatory responses at birth, this also shows a component in preventing chronic immune overreaction within immune disorders such as allergies. There is research shown that a deficiency or improper functioning of T-reg cells can increase risk of developing asthma (42, 43). There are many different theories regarding the cause of asthma

though research heavily suggests significance in improper immune functioning specifically regarding T cells.

Inflammatory bowel disease (IBD) refers to 2 separate conditions; Crohn's disease and ulcerative colitis are inflammatory conditions involving the gastrointestinal (GI) tract. IDB is considered an immune mediated inflammatory disease. Though the exact cause of IBD is unknown, it seems to be a multifactorial event causing the unnecessary inflammatory responses. Studies have shown T-cells play a significant role in the development of inflammatory bowel disease (IBD) (44). It is thought that mucosal CD4+ cells continued production of pro-inflammatory cytokines are responsible for the chronic inflammation seen with this disease (45). This chronic production of inflammatory cytokines stimulates the infiltration of other immune cells and labels the healthy tissue for destruction. There are also higher levels of IL-23 in those who have IBD (46). IL-23 promotes the stimulation and differentiation of T-cells which has the capability of provoking a large cascade of immune defenses in response. It has been shown that IL-23 can also suppress IL-10 (46,47) which is important in preventing an overwhelming inflammatory response. This also prevents the tissues damaged in these responses from being repaired by other cells. The challenge of identifying causes remains which can only be uncovered with additional research. Continued investigation of the early-life immune components and disorders that can be a of result of cell dysfunction is crucial in finding a cure.

Conclusion

The interplay of the immune system and all of the components required from an effective pathogen-clearing response creates many opportunities for complications to arise.

There are many downstream effects that take place with one simple reaction to an infectious component generates many avenues of malfunctioning of both innate and adaptive responses which can lead to complications later in life such as autoimmune disorders, cancers or even death. Fortunately, our bodies are always surveying and destroying errors that occur within these processes. The continued research of fetal immunity is necessary for finding future cures for autoimmune diseases via immune therapies.

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