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Fetal Regulatory T Cells and Peripheral Immune Tolerance *in utero*: Implications for Development and Disease

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Abstract

The developing fetus must actively learn to tolerate benign antigens, or suffer the consequences of broken tolerance. Tolerance of self-antigens prevents development of autoimmune diseases, and is achieved by both deletion of autoreactive T cell clones in the thymus (central tolerance) and by the suppressive influence of $CD4^+CD25^+FoxP3^+$ regulatory T cells (Tregs) in the periphery. Fetal $CD4^+$ T cells have a strong predisposition to differentiate into tolerogenic Tregs that actively promote self-tolerance, as well as tolerance to non-inherited antigens on chimeric maternal cells that reside in most fetal tissues. As the fetus nears birth, a crucial transition must occur between the tolerogenic fetal immune system and a more defensive adult-type immune system that is able to combat pathogens. This paper will review the unique tolerogenic nature of the human fetal immune system and will examine evidence for a novel model of fetal immune development: the layered immune system hypothesis.

Keywords

Fetal T Cells; FoxP3; Human Developmental Immunology; Layered Immune System; Microchimerism; Regulatory T Cells (Treg); Tolerance

Introduction

A developing mammalian fetus expresses a set of polymorphic major histocompatibility complex (MHC) molecules inherited from both its mother and father, meaning that up to half of those MHC molecules can potentially be recognized by the maternal immune system as allogeneic foreign tissue. A great deal of attention and thought has been given to the quandary of how the immune system of the mother deals with this antigen mismatch to avoid immune rejection of her developing fetus [recently reviewed by Chaouat et. al.][1–10]. Less scrutiny has been devoted to the reciprocal problem: how does the immune system of the fetus deal with the equally monumental challenge of developing in a semi-allogeneic host? One potential answer to this question is that the fetal adaptive immune system avoids rejection of the mother because it is inert, functionally impaired, and/or compromised due to limited antigen experience and a resultant insufficient cache of immunological memory. A historic and growing body of evidence that is discussed in this review argues that this hypothesis is not sufficient to explain fetal non-rejection of the maternal host. It is now clear that the human fetal immune system is highly active[11–20] but its activity cannot be defined entirely by traditional metrics of sterilizing immunity. Rather, we must consider the

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developmental state of the fetus to understand that its primary goal of its immune system is quite different: to achieve tolerance. The developing mammalian fetus exists in a relatively pathogen-free environment compared to the diverse microbial environment it will encounter after birth. This is not to say, however, that it is not exposed to or responsive against antigens. The fetal immune system must develop in such a way that it learns to tolerate benign and/or necessary antigens, including self-antigens, as well as environmental and food antigens that are transferred across the placenta. It is also now clear that hematopoietic cells of maternal origin are commonly found in tissues of the developing fetus, and several studies have shown persistence of maternal microchimerism even into adulthood[19, 21–25]. Given the genetic diversity of the polymorphic MHC locus, this means that up to half of the MHC antigens borne by maternal cells are different than those inherited by the fetus. If the fetus were to respond to these non-inherited maternal alloantigens (NIMAs) by generating a cytotoxic response, the result could theoretically be disastrous in that it could lead to fetal-anti-maternal alloimmune rejection and loss of pregnancy. To the contrary, however, there is now evidence that the fetus actively generates tolerance to these antigens, thereby avoiding a rejection response[19]. This review will focus on the best-defined mediator of this response, the fetal $CD4^+CD25^+FoxP3^+$ regulatory T cell (Treg), how it contributes to the fetal tolerance of NIMA, and what has been learned about fetal immune development from the study of fetal-maternal tolerance.

Mechanisms of fetal immune tolerance: central and peripheral

Throughout life, the acquired immune system must operate in balance between aggressive defense against potentially harmful invading pathogens and the tolerance of self-antigens and non-harmful commensal organisms. Furthermore, once a defensive immune response is initiated, mechanisms must come into effect to temper and eventually terminate that response lest it cause damage to host tissues resulting from ongoing and uncontrolled activation. A large body of literature demonstrates the unique nature of the developing fetal immune system and the importance of its unique ability to generate tolerance. In 1945, Ray Owen's studies in dizygotic freemartin twin cattle (which exchange blood during development in utero via placental anastomoses) provided early evidence that fetal exposure to non-self antigens results in enduring tolerance to those antigens. Though these twins are genetically non-identical, both autologous and non-self (twin-derived) erythrocytes can be persistently detected throughout the life of the animal, indicating an established immunological tolerance for the foreign antigens specific to the twin[26]. This observation was then extended to demonstrate that these animals are tolerant to post-natal transplantation of the twin's skin[27]. Experiments described by Billingham, Brent and Medawar in their seminal 1953 work, "Actively Acquired Tolerance of Foreign Cells" then went on to demonstrate that fetal exposure to antigenically dissimilar cells in fetal chicks and mice resulted in acquisition of enduring post-natal immune tolerance to tissues of the mismatched antigenic background. They showed that fetal exposure to foreign antigen lead to the absence of cells that would mount such an immune response, suggesting that elimination of such cells during development was the major mechanisms for establishing immune tolerance[27].

Central immune tolerance is orchestrated by the culling of autoreactive T cell precursors during differentiation in the thymus. Stochastic somatic rearrangement of T-cell receptor (TCR) genes in developing immature thymocytes results in a staggering diversity of potential T cell antigen specificities, many of which have potential to react against self-MHC or crucial structural, developmental, and/or functional proteins (e.g. insulin or collagen). To prevent these autoreactive cells from developing to maturity and causing damage, immature thymocytes traffic through the thymic medulla where they encounter self-antigen and other cross-presented antigens in the context of self-MHC. Those cells

whose TCRs bind avidly to self-antigen/MHC and transmit a strong signal through their T cell receptor then undergo apoptosis, leading to deletion of potentially autoreactive T cells. This process, known as clonal deletion, is also complemented by other mechanisms of eliminating autoreactive clones, including clonal diversion, TCR editing, and induction of anergy[28]. These first-line mechanisms to prevent anti-self immune reactions and autoimmune disease are crucial but not perfect, and autoreactive T cells can and do escape the thymus, as is evident in the case of autoimmune diseases that are driven by these cells. It is now clear, however, that periodic escape of autoreactive clones from thymic negative selection is likely the rule rather than the exception, even in the absence of clinical autoimmune disease. This is clearly demonstrated by experiments in which tissue-specific transgenic expression of co-stimulatory molecules and cytokines can result in antigen-specific immune attack of those tissues, meaning that mature lymphocytes escape clonal deletion and are capable of mediating reactions against self-antigen[29]. To deal with these rogue cells, a secondary line of defense exists in the form of active peripheral tolerance that is enforced by populations of regulatory cells with the ability to suppress potentially harmful autoimmune responses. The most extensively studied, and best understood, of these is the $CD4^+CD25^+FoxP3^+$ Treg. In the following section, their role in establishing and maintaining tolerance will be explored, including evidence that they contribute to prevention of autoimmune diseases.

Tregs and peripheral tolerance

Tregs play a crucial role in immunity as a ‘rheostat’ of the immune response: both preventing autoimmunity by inhibiting anti-self responses and also acting to suppress defensive immune responses at an appropriate stage to prevent host tissue damage. Basic molecular mechanisms governing the differentiation and function of Tregs have been recently reviewed by other authors and will not be discussed at length[30–36]. Is is important, however, to consider the role of Tregs in establishing and maintaining peripheral tolerance in order to understand their participation in fetal immunity. The existence of regulatory cell populations was suspected and pursued for many years, and the concept of the T suppressor cells was originally proposed and championed by Gershon and colleagues[37–40]. It is only in the last 10–15 years, however, that $CD4^+CD25^+FoxP3^+$ Tregs have been accepted as a crucial controller of the immune response. The initial discovery of Tregs came from experiments in which a specific population of $CD4^+$ cells that also highly express the high-affinity interleukin (IL)-2 receptor α-chain, CD25, were found to be protective in mouse models of autoimmunity[41–44]. It was subsequently shown that the transcription factor forkhead box P3 (FoxP3) was not only a specific marker for $CD4^+CD25^+$ Tregs, but was also crucial for their development, maintenance of phenotype, and function[45–51]. The importance of this factor came to light from both human clinical observations and genetic mutant mouse models which together demonstrated that disruption of the *FoxP3* gene results in an absence or paucity of regulatory cells leading to autoimmunity[45–58]. In 1982, a human syndrome was described that was defined by early neonatal onset in males of autoimmune disease in multiple organs, including thyroid, pancreas, gut, and skin[52]. The manifestations of the disease included type I diabetes, thyroiditis, inflammatory enteropathy, atopic dermatitis, and death from overwhelming infection, and the syndrome was named IPEX (for Immune Dysregulation, Polyendocrinopathy, Enteropathy, and X-linked). The syndrome was initially described as universally fatal, with decreased fetal viability or death within the first year of life[52]. Meanwhile, a mouse strain called *scrufy* was identified as a spontaneously arising mutant with a strikingly similar phenotype to patients with IPEX[54–56, 58]. Hemizygous *scurfy* males die within the first three weeks after birth with disease characterized by T cell over-proliferation and extensive multi-organ leukocyte infiltration and autoimmunity[53–56]. The gene defective in the scurfy mouse was mapped to the *FoxP3* locus, and genetic complementation with *FoxP3* rescued the scurfy phenotype[46].

It was subsequently demonstrated that induced disruption of *FoxP3* resulted in the absence of Tregs and reproduced the characteristics of the scurfy phenotype[48]. *FoxP3* is strongly conserved between mice and humans, and subsequent studies confirmed that *FoxP3* disruption and the consequent absence of Tregs is the primary immune lesion in IPEX[45, 57, 58].

The mechanisms by which Tregs function to suppress immune responses have been intensely studied, and there seem to be diverse Treg responses that come into play depending on the context of activation and the environment in which they are operating[59, 60]. Specifically, the mechanisms of Treg-mediated suppression seem to be determined at least in part by whether they are maintaining immune quiescence to prevent immune activation in the physiological homeostatic steady state or are responding to dampen an active inflammatory response[61]. The mechanisms used by Tregs to suppress immune responses include: transmission of inhibitory signals via cell-cell surface interactions or secreted cytokines, diminishing conventional T cell activation or fitness by limiting growth factors like IL-2 or essential amino acids, direct target-cell cytotoxicity, and/or modulation of antigen presenting cell function[36, 59–61]. Like other αβ TCR-utilizing T cells, Tregs have a diverse TCR repertoire and can respond to a wide range of antigens. Though they do not seem to have an absolute requirement for recognition of specific self-antigens to mediate suppression, clonal Treg pools responding against a specific antigen recognized by their TCRs seem to be more effective suppressors than polyclonal populations mediating non-specific suppression[62–65].

In the years since their discovery and acceptance as being functional regulatory cells, it has become clear that Tregs play a crucial role in maintaining peripheral tolerance and immune homeostasis. Insufficient or dysfunctional Treg responses are thought to contribute to the pathogenesis of several disease states resulting from broken self-tolerance, including Type I diabetes[63, 65–67]. Not only are Tregs a dominant mediator of peripheral self-tolerance, they also appear to be important in modulating the innate and acquired immune responses to foreign antigen[68–71]. Most circulating Tregs differentiate from T cell precursors in the thymus, and are thereafter phenotypically and functionally distinctive compared to conventional $FoxP3^{-}CD4^{+}$ T cells[36, 72]. These ‘thymic’ or ‘natural’ Tregs (nTreg) likely play a crucial role in maintenance of tolerance to self-antigen, and to other antigens presented in the thymus. Tregs can also, however, be generated under specific circumstances from $FoxP3^{-}CD4^{+}$ conventional T cells after thymic egress[73–82]. These cells have been called ‘peripheral’ or ‘induced’ Treg (iTreg), and may play a role more in the tempering of responses to antigens not encountered in the thymus, including pathogen-related antigens. One of the best-studied iTreg populations resides in the colon, develops in response to commensal bacteria, and is thought to be important for maintenance of tolerance to these commensals[68, 70]. Differentiation or ‘conversion’ of conventional Tregs into iTregs is dependent on several factors, including the strength of antigen signal, specific signals from antigen presenting cells, and the nature of the local cytokine milieu. In particular, activation of T cells under the influence of transforming growth factor-β (TGF-β) and IL-2 can induce naïve CD4+ T cells to induce FoxP3 expression and adopt a regulatory phenotype[83–85]. The role of Tregs in promoting both solid organ and hematopoietic transplantation tolerance has also been of great interest[71]. Infusion of Tregs that are polyclonally expanded *ex vivo* is now being studied in phase I and II clinical trials of hematopoietic stem cell transplantation, and demonstrates great promise as a strategy to reduced graft-versus host disease[69, 71, 86, 87]. This begs the important question whether Tregs might play a role in establishing and maintaining tolerance to the only naturally occurring allograft encountered by the immune system: the fetus.

Tregs contribute to maternal tolerance of the fetus

First clearly demonstrated by Alexander Betz, and colleagues a significant body of evidence from mouse models now demonstrates that maternal Tregs that are induced by, and are reactive against, paternal alloantigen contribute to successful implantation and maintenance of pregnancy[7, 88–98]. Though challenging to demonstrate definitively, it also now seems that maternal Tregs also play a similar role in protecting the fetus from rejection during human pregnancy[93, 95, 99–102]. During normal early (5–9 gestational weeks) human pregnancy, Sasaki et al. observed that suppressive $CD4^{+}CD25^{high}$ T cells are enriched in the decidua, and that this enrichment was not found in cases of spontaneous abortion[100]. Similarly, the frequency of $CD4^{+}CD25^{high}$ T cells was found to increase in the peripheral blood of pregnant women at increasing frequencies that peaked in the second trimester and declined after birth[99]. More recent evidence suggests that maternal failure to increase circulating Treg frequency is correlated with pregnancy loss[102]. It has also been shown that in preeclampsia there is also a relative failure to increase circulating Tregs, leading to a relative imbalance of regulatory and inflammatory cells compared to normal pregnancy[101]. Together, these observations suggest that maternal Treg responses in humans also contribute to maternal tolerance of fetus. The mother may also be primed toward tolerance by exposure to paternal antigens in the context of immunomodulatory factors in semen or at the utero-placental interface[1–6, 103–108]. It is also clear, though, that cells from the fetus transit into the mother, establishing residence in maternal tissues and resulting in microchimerism that can last for many years[22, 24, 25]. These microchimeric cells likely provide a stimulus for initiation and generation of the tolerogenic Treg response demonstrated in pregnancy. Immune chimerism also occurs in the opposite direction, and maternal cells have been found to routinely (if not universally) reside in fetal tissues[19, 21–25]. This begs the question: why does the fetus not reject the mother? Work by many investigators has demonstrated that the human fetal peripheral immune system is highly active, and that fetal T cells are intrinsically capable of becoming activated in response to foreign antigen[11–20]. Could this then suggest that active peripheral tolerogenic mechanisms might contribute to the physiological absence of fetal anti-maternal rejection?

Part of the challenge in studying fetal immunity has to do with the diversity and variability in immune development between species, and especially the difference between humans and laboratory mice. Inbred mouse strains commonly used in the laboratory begin to populate the thymus with T cell precursors at about 12 days of gestation[109]. The developing fetal mouse does not start to populate secondary lymphoid structures until near the end of gestation, making the newborn mouse more closely resemble a human at a much early fetal developmental stage[110, 111]. T cells are absent in the newborn mouse spleen and are very sparse in the lymph nodes, where they have a primitive, simplified spatial organization[110]. During the first week of life, lymph node T cells become more abundant and adopt a more mature architecture, while the spleen becomes populated with T cells[110]. Tregs typically do not exit the thymus in mice until at least three days after birth, and increase to adult levels over three weeks[112]. This developmental schedule would lend support to the assumption that the fetus was not significantly challenged by maternal antigen in the immune periphery, and that tolerance of self-antigen in the developing fetus was entirely due to central deletion of autoreactive cells. The human fetal schedule of immune development is highly distinct from the mouse however, and suggests the need for active peripheral tolerance mechanisms. In the human fetus, T cell precursors transit to the thymus by 9 weeks of gestation[14]. Mature naïve and memory αβ T cells are readily found by 12–14 weeks in spleen and lymph nodes, and are abundant by the end of the second trimester[11–14, 18, 19]. Impaired fetal survival and early demise in IPEX syndrome clearly demonstrates the clinical importance of a peripheral tolerogenic mechanism in human immune development. Another compelling hint that the human fetal immune system may actively generate tolerance came from the studies of

Burlingham and colleagues, who demonstrated that long-term survival of kidney grafts was enhanced when the donor carried mismatched human leukocyte antigens (HLA) that were the same as non-inherited alleles from the recipient's mother (i.e. NIMAs)[113]. Although the recipient had no opportunity to generate post-natal tolerance to these unshared maternal antigens, there was nevertheless some factor preventing their rejection that was likely to have arisen during the period of exposure to NIMAs in utero. This finding suggested a fundamental shift in the understanding of how the fetal immune system develops, and implied that humans have the ability to generate active, long-lasting, post-natal tolerance to antigens experienced in utero.

Tregs are abundant in the developing human fetus

The developing human fetal immune system in the second trimester is distinct from that of the newborn, or the mature adult. One of the most striking differences is that fetal tissues have an increased frequency of Tregs compared to any other time in development[16, 18, 19, 114]. The earliest observation of this phenomenon may have been in the work of Cooper and colleagues, who demonstrated that fetal spleen and premature cord blood were enriched for a $CD45RO^+$ memory population that also expressed CD25, was highly proliferative in response to IL-2, but not to standard mitogenic stimuli (e.g. anti-CD3)[15]. After the description of Tregs, and FoxP3 as their definitive marker, it was confirmed that secondary fetal lymphoid tissues in the second trimester contain a surprisingly high abundance of $CD4^+CD25^+FoxP3^+$ Tregs, on average 15–20% of $CD4^+$ cells[16, 18]. This is in contrast to full-term umbilical cord blood, adult peripheral blood, or lymph nodes from adults, in which Tregs typically represent less than 5% of total $CD4^+$ cells[18]. The abundance of Tregs in secondary fetal lymphoid organs was not reflected in the thymus at equivalent gestational ages, where the frequency of $CD25^+FoxP3^+$ single CD4 (sp4) thymocytes was comparable to the infant thymus (about 10–12%)[18]. This suggested that a significant proportion of the Treg-enriched fetal T cell population consisted of Tregs that expanded from nTregs, or were generated from conventional $CD4^+FoxP3^-$ T cells, in response to antigen. Upon depletion of $CD25^+$ T cells from fetal lymph node-derived T cells, a significant proportion of the remaining conventional T cells both (1) proliferate spontaneously; and (2) produce interferon-γ (IFN-γ) in response to SEB stimulation (which they did not in the presence of Tregs)[18]. These findings demonstrated the suppressive influence of Tregs in human fetal lymph nodes, and implied that the fetal immune system may have the ability to generate active peripheral tolerance via these cells

Fetal Tregs generate specific tolerance toward maternal alloantigen

Given that a significant frequency of human T cells have the capacity to recognize alloantigen, the fetal immune response against NIMA was used as an *in vivo* model system to test the hypothesis that fetal Tregs are generated from conventional fetal T cells in response to antigen stimulation.[19] Maternal cells were confirmed to be in relatively high abundance (up to 0.8%) in second-trimester fetal lymph nodes, and cells were from each major hematopoietic lineage were found to be present in full-term umbilical cord blood (including T, B, NK, and monocytes)[19]. In mixed lymphocyte reaction (MLRs), fetal immune responses against maternal antigen presenting cells (APCs) bearing NIMAs were dampened compared to responses against unrelated alloantigen from third-party donors[19]. This demonstrated that fetuses are more tolerant toward NIMAs, and knowing that they are exposed to those antigens on resident maternal cells, it was proposed that this tolerance was mediated not only by central clonal deletion, but also by peripherally generated Tregs. Fetal T cells that were depleted of $CD25^+$ Tregs prior to MLR had significantly enhanced immune reactions against self and maternal APC, but not against unrelated donors, confirming the presence of NIMA-specific Tregs[19]. It was unknown whether these were nTregs generated

against maternal antigen presented in the thymus, or if iTregs generated in the periphery in response to stimulation by maternal cells in secondary lymphoid organs may contribute to anti-NIMA tolerance. To determine if these cells could be generated from non-Tregs, conventional FoxP3^{-} naïve (CD45RA^{+}CCR7^{+}) T cells were stimulated with foreign APCs. Surprisingly, alloantigen-stimulated fetal T cells overwhelmingly differentiated into CD25^{+}FoxP3^{+} Tregs that were confirmed to mediate alloantigen-specific suppression[19]. This effect was dependent on TGFβ signaling, and fetal lymph nodes were found to express significantly higher levels of TGF-β family members compared to adult lymph nodes. These findings do not rule out the possibility that fetal Tregs represent a mixed population of nTregs and iTregs, but do clearly demonstrate that fetal T cells have a strong propensity to become functional tolerogenic Tregs upon antigen stimulation, and that the fetal peripheral immune niche is tuned to support such a response.

Implications for fetal and neonatal infection

The strong predisposition of fetal T cells to differentiate into Tregs has many potential implications for the overall function of the fetal immune response and the nature of its interactions with both benign (self, maternal, environmental, commensal microbial, and food) antigens as well as antigens associated with potentially harmful pathogens. Given that the in utero environment is relatively protected against microbial infection, it makes teleological and evolutionary sense that T cells in the developing fetus may be predisposed to mount tolerogenic responses, and that the niche in which they develop may support such responses. As seen in the example of IPEX, the absence of such tolerance is disastrous[52]. In the face of microbial or viral infection, however, a dominant tolerogenic response might theoretically be detrimental. This raises an interesting possibility that the fetal predisposition toward tolerance could contribute to the enhanced susceptibility to serious infection that is well recognized in fetuses and newborns, and particularly in premature newborns.

Infection is a leading cause of death and morbidity in newborns. Not only are neonates susceptible to more severe forms of disease caused by typical human pathogens, they are also subject to serious infection by microbes that are considered commensal flora in adults. For example, even after implementation of intensive screening and prevention practices, the estimated rate of Group B Streptococcal sepsis in the first week of life is 0.34 per 1000 live births, resulting in 60–70 deaths per year in the United States alone[115]. Premature infants are especially predisposed to more severe infections from all pathogens and can also succumb to fatal infection by microbes that infrequently cause severe disease in adults[116]. This increased susceptibility to infection is accompanied by a relatively ineffective response to neonatal vaccination[117, 118]. Compared to adults and older children, even full-term newborns produce less, and generally less effective, antibody in response to most immunizations. They are also less able to generate effector T cells that mediate effective antimicrobial responses[117, 119–122]. Together, these deficiencies render the fetus and neonate a vulnerable target for a host of invading pathogens. Many mechanisms of classical host defense are compromised in the fetus and neonate, but this has often been attributed to the immature developmental state of the immune system, or to the absence of antigen exposure. The work discussed here demonstrates that the fetal immune system is most certainly not inert, but rather is extremely active and capable of responding to antigenic stimulation. The nature of its response, however, while appropriately developmentally tuned to create tolerance, may predispose the fetus toward tolerance of harmful microbes in the face of infection. The negative implications of this tolerance-promoting mandate are clear: when faced with an invading microbe, the human fetal tendency to generate tolerance to antigens associated with that microbe may be detrimental if it allows infection to proceed unchecked. This hypothetical explanation for fetal and neonatal susceptibility to infection remains to be tested, but does provide a novel model with which to frame such pressing questions. It also

raises the important question of how a tolerogenic immune program that is crucial for survival and development in utero is ultimately converted into an immune response that can functionally meet the outside world.

A (not-so) new model for fetal immune development: the layered immune system

As the fetus nears birth, the tolerogenic fetal T cell response must be converted into a response dominated by T cells capable of supporting defensive antimicrobial immunity. To begin to understand this process, and the mechanisms by which it might be governed, it was necessary to delve further into the nature of the fetal immune system, and how it might be different from that of the adult. Global gene expression profiling on human fetal and adult $CD4^+$ naïve T cells and Tregs followed by comparative bioinformatic analysis revealed that phenotypically similar T cells in the fetus and adult had strikingly different gene expression profiles[123]. This was true of both Tregs and naïve T cells, and many of the genes that were differentially expressed in fetal naïve T cells were also similarly differentially expressed in fetal regulatory T cells. The commonality of fetal naïve and Treg gene expression profiles suggested that fetal T cells could represent a unique hematopoietic lineage. There is, in fact, strong historical evidence in both avian and mouse models that fetal hematopoietic stem-progenitor cells (HSPCs) can give rise to unique subsets of lymphocytes in the fetus that cannot be generated from adult HSPCs[124–132]. In the mouse, the first wave of T cell progenitors that colonize the fetal thymus differentiate into a unique fetal subset of γδ TCR-utilizing T cells with a restricted Vγ3/Vδ1 TCR[125]. These cells are eventually replaced by a wave of more diverse γδ T cells and ultimately, prior to birth, by a wave of T cells utilizing the αβ TCR[125, 129]. This phenomenon echoes earlier reports in quail/chick chimeras in which three waves of thymocytes populate the thymus, and replace one another in sequence[133, 134]. A similar phenomenon has been described and well characterized in mouse B lymphocyte development. Two distinct lineages can be defined based on surface phenotype staining, the B-1 and B-2 lineage, initially defined by CD5 (Ly-1) expression[135, 136]. The B-1 lineage seems to be a more primitive one that is found in newborn mice and cannot be efficiently generated by adoptive transfer of adult bone marrow[128]. Together, these findings suggest a model whereby maturation of progeny cells derived from distinct hematopoietic progenitors results in waves of mature immune effector cells that are tuned to the specific developmental needs of the organism. As these progressive, distinct waves accumulate, they co-exist for a period of time and result in ‘layers’ of immune cell lineages. This model, first formally proposed by Lee and Len Herzenberg, has become known as the layered model of immune development (Figure 1)[137]. Given the highly unique gene-expression profile of fetal T cells (including fetal Tregs), it stood to reason that these cells could represent their own wave of T cell progeny arising from a distinct fetal hematopoietic progenitor.

To test the hypothesis that fetal T cells represent a unique cell lineage compared to adult T cells, a series of experiments were carried out in which fetal HSPCs from human fetal liver and bone marrow (18–22 gestational weeks) and adult bone marrow were injected directly into the human Thy/Liv organ of the SCID-hu Thy/Liv mouse[123]. This humanized mouse model allows for reproducible, multi-lineage hematopoiesis (including thymopoiesis) from human HSPCs[138, 139]. Global gene expression analysis was carried out on mature $CD3^+CD4^+CD8^-CD25^-$ sp4 thymocytes that were differentiated from fetal liver-, fetal bone marrow-, or adult bone marrow-derived HSPCs using this model. Surprisingly, HSPCs from both fetal liver and bone marrow gave rise to identical populations of sp4 thymocytes on the basis of gene expression. By contrast, adult bone marrow-derived HSPCs had a radically different gene expression profile compared to each population of fetal HSPC-derived thymocytes. There was significant overlap between genes expressed in primary peripheral

$CD4^+$ fetal T cells and $CD4^+$ thymocytes derived from fetal HSPC, and the same was true of adult peripheral $CD4^+$ T cells and $CD4^+$ thymocytes derived from adult HSPC. This strongly suggested that fetal and adult HSPCs give rise to developmentally unique lineages of fetal T cells, thus, providing strong evidence that the layered immune system hypothesis can be extended to describe human T cell development. Considered in context, this hypothesis proposes that fetal immune development proceeds in such a manner that a dominant tolerogenic fetal immune system is progressively replaced by an immunogenic adult-type immune system, resulting in co-existing populations (or layers) of tolerogenic and immunogenic T cells. These two immune systems serve different roles based on developmental state of the organism, and the sum immune response that is generated toward antigenic stimulation is the result of the relative contribution of these two opposing T cell layers. Future perspectives for peripheral fetal tolerance and the layered immune system hypothesis.

The layered immune system hypothesis may have potential to enhance our understanding of normal fetal immune development, and also represents a new way to model the pathophysiology of many diseases. For example, if the type and magnitude of response that is generated in utero or at birth is determined in part by the opposing fetal and adult influences, the relative contribution of each may determine whether the ultimate response is tolerogenic, immunogenic, or an intermediate of the two. Within a human population, if there is variability in the degree to which layering occurs at birth, this could theoretically also lead to equal variability in the nature of immune responses that can be generated. As an example, consider the scenario in which the transition from fetal to adult T cell predominance is delayed compared to the population norm, resulting in a newborn infant with an over-representation of fetal T cells. When faced with colonizing microbes, including GBS, this infant might be more predisposed to mount a tolerogenic response to those microbes. In that scenario, an organism that would normally become a commensal symbiotic microbe in most infants could potentially cause invasive infection and serious disease. Conversely, an infant who had precocious maturation of the adult compartment may then have over-representation of adult T cells at birth. Such an infant may then be at risk of overly zealous immune responses against normally tolerated antigens such as self, environmental, or food antigens, potentially leading to auto-immune disease, allergy, or food-antigen intolerance. To address these questions, it will be necessary to better understand the timing of, and mechanisms governing, the transition from tolerogenic fetal responses to immunogenic adult responses.

Another question to be answered is whether hematopoiesis arising from fetal HSPCs persists throughout post-natal life, and whether T cells arising from these progenitors play a role in homeostasis, health, and disease. Tregs capable of mediating specific tolerance to NIMA persist in children and young adults[19]. It is not clear whether these Tregs represent long-lived cells acquired during fetal development, the progeny of such cells, or Tregs arising more recently due to persistence of chimeric maternal cells in the offspring. It is intriguing to consider that fetal stem-progenitor cells (or their long-lived progeny) may persist in a normally non-dominant state throughout life, and could potentially be re-activated in situations where re-acquisition of tolerance is needed (e.g. after non-ablative chemotherapy, or during immune reconstitution after initiation of antiretroviral therapy for HIV disease). This possibility also begs the question whether, in some cases, broken or incomplete tolerance (e.g. autoimmune diseases) represents the failure of a tolerogenic fetal T cell population. Though these considerations remain hypothetical, ongoing investigations will hopefully lead to further insights into the process of normal human fetal immune development and its influence of on how our immune systems learn to interact with ourselves, and the environment in which we exist.

Summary

In conclusion, the immune environment of the developing human fetus is especially well suited to generate peripheral tolerance. Fetal T cells, which are derived from a developmentally restricted hematopoietic lineage, appear to be a major player in this tolerogenic disposition because they are relatively enriched in Tregs, and there is a strong tendency for naïve CD4+ fetal T cells to differentiate into Tregs upon antigen stimulation. These fetal Tregs are capable of quelling immune responses against maternal alloantigen, and may therefore provide a means by which the fetus prevents maternal rejection to allow for maintenance of pregnancy. A deeper understanding of the nature of the developing human fetal immune system has profound potential to help us understand health and disease in the fetus, newborn, and likely every stage of life thereafter.

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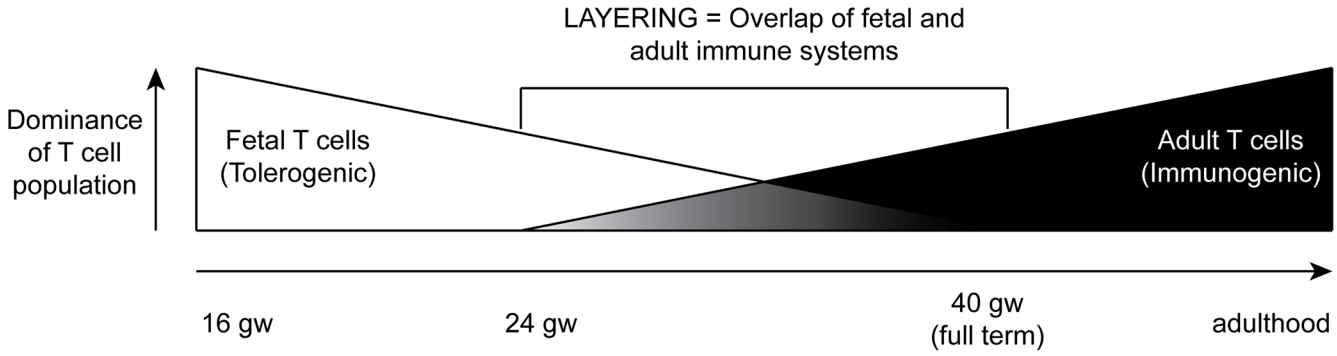

Figure 1.

Representation of the layered immune system in the human fetus. Tolerogenic fetal T cells derived from fetal hematopoietic stem–progenitor cells (HSPC) dominate fetal immune responses until the third trimester, when a population of adult HSPC-derived immunogenic T cells come to dominance. During the transition period, a layered immune system occurs, and the prevailing nature of the immune response may be governed, in part, by the degree of layering of fetal and adult T cells and the relative influence they exert.