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Development of the early immune system: impact on allergic diseases

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The advent of molecular tools, transgenic models, and genomic approaches has resulted in an explosive increase in the understanding of the immune system. This article considers how advances in the understanding of innate and adaptive immunity have crafted the knowledge of the fetal immune system, this system's interaction with the maternal environment, and the impact on the development of allergic diseases. Future examination of these pathways, their intersection with environmental exposure, and the interactions between the innate and adaptive immune systems may provide attractive therapeutic approaches for the treatment and prevention of allergic diseases.

The incidence of allergic diseases, especially allergic asthma, is increasing. Although genetic and environmental factors contribute to the pathogenesis of these diseases, the molecular pathways induced by these factors in humans have not been well defined. Increasingly, the factors leading to the initiation and maintenance of the asthmatic inflammatory response are presumed to begin in early life, even in utero. Regardless of the inciting agents, asthma is an inflammatory disease that predominantly involves the activation of adaptive immune pathways. Epidemiologic studies suggest that the innate immune system has important interactions with the adaptive immune system in the development of allergic diseases. Normal immunity must be understood before therapeutic strategies can be developed to prevent or control exuberant or dysfunctional immunity. This article focuses on advances in the understanding of the early development of the immune system and highlights the knowledge of the interaction between the innate and adaptive immune systems in the development of allergic diseases.

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Innate versus adaptive immunity

The immune system is composed of two elements—the innate system and the adaptive system. These systems have evolved independently over time and serve unique functions in the immune response. The adaptive immune system is characterized more fully than is the innate system. Great strides have been made toward elucidating the role of innate immunity as the body's first line of defense against a foreign invader. Innate immunity is activated immediately, without need for specificity, to combat infection or other danger signals [1]. Because of the observation that infections in early life are associated with the later onset of asthma [2–4], this section focuses on infection and the innate immune response.

Innate immunity

The innate immune system developed before the evolutionary separation of vertebrates from invertebrates [5]. Unlike recognition of a pathogen by the adaptive immune system, which is commanded by antigen-antibody specificity, recognition by the innate system is predetermined, obviating the need for previous exposure.

The adaptive immune system relies on B and T lymphocytes and their unique antigen specificities. Distinct receptors on lymphocytes are generated through genetic recombination of gene segments, which encode part of the variable region of one of the immunoglobulin polypeptide chains. Hundreds of different gene segments produce thousands of different receptor chains through varied combination. At any given time in a human, lymphocytes have at least 10^8 different specificities [6]. The innate system, however, recognizes only a number of patterns [7].

The innate system exerts broad impact by targeting patterns common to a large number of microorganisms. Many microorganisms are linked by common molecular patterns, called pathogen-associated molecular patterns (PAMPs) [8], that are recognized by the pattern-recognition receptors (PRRs) of the innate immune system [9].

PAMPs

Pathogen-associated molecular patterns include fungal β -1, 3-glucan, bacterial lipopolysaccharide (LPS) and peptidoglycan, phosphoglycans, lipoteichoic acids, mannans, bacterial DNA, and double-stranded RNA. [5, 7]. These molecular patterns are often central to the survival or pathogenicity of the invading pathogen. Because gram-negative bacteria contain LPS, gram-positive bacteria contain lipoteichoic acids, and fungi contain mannans, the innate system is pervasive, attacking entire classes of microorganisms despite a limited number of receptors [5,8].

PRRs

Pattern recognition receptors are located on B cells, dendritic cells, and macrophages. These proteins exist not only on the cell surface but also in the cytoplasm and as secreted proteins [8]. Leucine-rich repeats, calcium-dependent lectin, and cysteine-rich scavenger receptors are protein domains common to PRRs [7]. PRRs have distinct functions: secreted PRRs act as opsonins, PRRs on the phagocyte cell surface mediate endocytosis, and still others activate signal-transduction pathways involved in inflammation [7].

Opsonins

A well-characterized example of a PRR that acts as an opsonin is the mannose-binding protein (MBP). This protein is synthesized in the liver and is released as an acute phase response to a pathogen. MBP is a calcium-dependent lectin receptor that binds to mannose-rich carbohydrates on gram-positive bacteria, gram-negative bacteria, yeast, and some viruses and parasites. In humans, the secreted MBP serves as an opsonin in serum, enhancing phagocytosis of mannose-rich pathogens, such as *Salmonella* [10,11]. MBP triggers complement activation through classical and alternate pathways [12,13].

Endocytic receptors

The mannose receptor, an endocytic PRR, can recognize and internalize organisms, even in the absence of opsonins. In mice, it exists on macrophages, dendritic cells, and some endothelial cells [14]. Of clinical relevance, the mannose receptor mediates phagocytosis of *Pneumocystis carinii* [15] by alveolar macrophages [16]. Investigators have isolated alveolar macrophages from healthy volunteers and patients with HIV. The patients with HIV were grouped into those with a CD4 cell count less than 200 cells/mm³, a CD4 cell count greater than 200 cells/mm³, or active *P. carinii* pneumonia (PCP). In macrophages from patients with HIV, surface expression of the mannose receptor and endocytosis were reduced by up to 80%, in part because of a reduction in levels of mannose-receptor mRNA. Patients with HIV and CD4 cell counts less than 200 cells/mm³ had the lowest levels of phagocytosis and mannose-receptor endocytosis of *P. carinii*. This observation was attributed to a significant reduction in the number of organisms associated with each macrophage. Infection of normal alveolar macrophages with HIV in vitro resulted in a significant reduction in mannose-receptor endocytosis, *P. carinii* binding, and phagocytosis compared with that in controls. These results imply that HIV may impair the ability of the alveolar macrophage to combat HIV-related PCP by changing the mannose-binding receptor [17].

Toll-like receptors and lipopolysaccharide: activation pathways

Recognition of PAMPs by a signaling receptor leads to activation of signal-transduction pathways. Such pathways are responsible for the production of cytokines and the activation of genes involved in inflammation. One such receptor,

the human Toll-like receptor, first was characterized in the fruit fly, which emphasizes how the ancient innate immune system relates vertebrates to invertebrates [5]. The *Drosophila* Toll protein is a transmembrane receptor with extracellular, leucine-rich repeats and a cytoplasmic signaling domain. The cytoplasmic domain is homologous to the cytoplasmic domain of the interleukin 1 receptor (IL-1R). The Toll protein in *Drosophila* and IL-1R in humans can stimulate signal transduction pathways, which induce the activation of transcription factors such as nuclear factor- κ B (NF- κ B) [18].

The human correlate of the *Drosophila* Toll protein is the Toll-like receptor 4 (TLR4), which has an extracellular domain composed of leucine-rich repeats and a cytoplasmic domain that is similar to the intracellular domain of the IL-1R. A constitutively active human Toll protein that is transfected into human cell lines induces genes that code for the proinflammatory cytokines IL-1 and IL-8 and the costimulatory molecule B7-1 (CD80). Induction of these effector cytokines links the innate immune system to the adaptive immune system [19].

Lipopolysaccharide, an endotoxin that is common to gram-negative bacteria, is recognized by TLR4. LPS and TLR4 genes have been localized to the same region on mouse chromosome 4 [20]. In murine models, mutations in the TLR4 gene lead to hyporesponsiveness or tolerance to endotoxin [20,21]. Approximately 10 mammalian Toll receptors have been identified. These Toll proteins seem to recognize discrete molecules that are common to microbes. TLR4 is crucial for the recognition of gram-negative infection [22], TLR2 is important for the recognition of gram-positive and mycobacterial infection [23,24], and TLR9 is important for the recognition of bacterial cytidine-guanosine dinucleotide (CpG DNA) [25].

Toll-like receptor 4 does not act alone in the recognition of LPS. LPS first encounters the acute-phase reactant LPS-binding protein [26], which transfers LPS to CD14 [27]. CD14 exists as a receptor on monocytes and macrophages. Pretreatment of monocytes with a monoclonal antibody to CD14 leads to significant inhibition of T-cell proliferation that is induced by LPS-primed monocytes [28]. A third protein that is closely associated with TLR4 on the cell surface, named MD-2, is believed to stabilize the receptor complex and is required for complete recognition of LPS [29]. LPS-induced T-cell proliferation is dependent on cell-to-cell contact between monocytes and T lymphocytes [30]. Cell-to-cell contact allows receptor-ligand interactions between the costimulatory molecules CD28 and CTLA-4, and CD80 and CD86 [28], which are described in the section on adaptive immunity.

Lipopolysaccharide induces the expression of costimulatory molecules and up-regulates an activating receptor of the immunoglobulin superfamily expressed on myeloid cells called TREM-1 (triggering receptor expressed on myeloid cells 1) [31]. This receptor is expressed preferentially on neutrophils and monocytes and is a transmembrane receptor with a short intracytoplasmic tail that is incapable of transducing a signal [32]. TREM-1 associates with a separate adapter protein of the signal transduction transmembrane called DAP12 [31]. DAP12 contains a cytoplasmic immunoreceptor tyrosine-based activation motif (ITAM). Once ITAM is phosphorylated, protein tyrosine kinases are recruited to initiate further phospho-

rylation, which leads to cellular activation. Expression of TREM-1 is up-regulated specifically by extracellular bacteria (eg, *Pseudomonas aeruginosa*, *Staphylococcus aureus*), LPS, and lipoteichoic acid. TREM-1 is not up-regulated in nonmicrobial inflammation. Studies suggest that treatment with a fusion protein (murine TREM-1 extracellular domain and human immunoglobulin- γ [mTREM-1 [IgG1] Fc portion IgG1]) improved the survival rate from endotoxemia in LPS-challenged mice. This effect was still protective when the fusion protein was administered up to 6 hours after LPS exposure. These data suggest that blockade of TREM-1 may have a therapeutic benefit after the onset of infection [32].

Innate immunity and pulmonary host defense

With every breath, the surface area of the lung is exposed to airborne particles and foreign invaders. The immediate innate immune system is a key element in pulmonary host defense. Particles less than 5 μm in diameter can access the lower airway, where they encounter a variety of antimicrobial molecules of the innate immune defense. These molecules include lysozymes, IgA, IgG, complement, LPS-binding protein, fibronectin, transferrin, lactoferrin, defensins, cathelicidins, and collectins [33].

Collectins: surfactant proteins

Surfactant proteins A and D (SP-A, SP-D) are members of the collectin family, which are important factors in human host defense. Both proteins contain collagen and lectin-like domains. The genes that encode these proteins have been localized to the region of chromosome 10q22.2-23.1 [34] near the locus for MBP [35]. They bind a variety of carbohydrates, carbohydrate-containing ligands, and lipids [36]. Surfactant proteins defend the host against microbes by acting as opsonins and by stimulating phagocytosis by rat alveolar macrophages [37–39]. Some controversy exists regarding the action of surfactant proteins, because simultaneous additions of SP-A and LPS to bacteria also have inhibited phagocytosis by alveolar macrophages [40].

Surfactant proteins have been shown to affect T-lymphocyte proliferation and cytokine production. Yao and colleagues described the effect of small aggregate surfactant subfractions from rats on mitogen-stimulated proliferative responses of human peripheral blood mononuclear cells (PBMCs). Lower concentrations of small aggregate surfactant subfractions resulted in a two-fold increase in lymphocyte proliferation, whereas higher concentrations inhibited lymphocyte proliferation, indicating a potential concentration-dependent effect [41]. SP-A also has been shown to increase human PBMC secretion of tumor necrosis factor α (TNF- α), IL-1 α , IL-1 β , and IL-6. Increasing amounts of surfactant lipids attenuate the increase in levels of TNF- α in response to SP-A [42], implying potential counter-regulatory roles of SP-A and surfactant lipids. Surfactant phospholipids also have been shown to suppress mitogen-induced lymphocyte proliferation. This suppression can be reversed by addition of SP-A, which stimulates proliferation [43]. The varied surfactant concentration and composition

that occurs during pulmonary inflammation or infection may modulate immune cell function by regulating lymphocyte proliferation and cytokine production.

The action of surfactant proteins is not limited to microbes; they also bind pollen, Kentucky blue grass, cultivated rye, and short ragweed by way of the lectin domain [44]. Similarly, SP-A and SP-D from human bronchoalveolar lavage fluid bind whole-mite extracts (*Dermatophagoides pteronyssinus*) and purified allergen Der p 1 by way of a carbohydrate residue–receptor interaction [45]. Systemic sensitization to ovalbumin (OVA) followed by aerosolized challenge resulted in eosinophilic pulmonary infiltrates, increased airway hyper-responsiveness (AHR), and increased expression of SP-A and SP-D within bronchiolar cells in an in vivo murine model of asthma [46]. These observations illustrate a role for surfactant proteins in the pulmonary allergic response.

The innate immune system is an ancient form of defense against many types of foreign infectious pathogens. Innate immunity acts immediately in a non-antigen-dependent fashion, permitting the development of adaptive, cell-mediated immunity while protecting the host.

Adaptive immunity

The hallmarks of the adaptive immune system are the ability to learn, gain memory, and obtain specificity, which are the primary features that distinguish this system from the innate immune system. The components of the adaptive immune system are humoral (eg, immunoglobulins) and cellular (eg, T cells, B cells, antigen-presenting cells [APCs]). To mount a full immune response, the adaptive immune system must be primed for the receptor-specific T cell to proliferate (clonal selection theory). In addition to its role in defense mechanisms, this system is critically involved in autoimmune and allergic diseases.

Role of T cells in allergic diseases

Adaptive immunity, unlike innate immunity, is antigen dependent. T cells recognize specific antigens, which are presented by APCs by way of major histocompatibility complex class I (MHC I) or class II (MHC II) molecules to the T-cell receptor (TCR). The TCR is composed of either an α -chain and a β -chain or a γ -chain and a δ -chain, which are associated with four proteins. Whereas α/β -T cells are predominant in the thymus, circulating blood, lymph nodes, and spleen, γ/δ -T cells are distributed in the tissues. Association of the TCR with the CD3 complex facilitates signal transduction through phosphorylation and activation of protein tyrosine kinases. Depending on the type of MHC, either CD8⁺ T cells (by way of MHC I) or CD4⁺ T cells (by way of MHC II) are activated.

T cells, B cells, and natural killer (NK) cells are derived from a common lymphoid progenitor cell. This cell gives rise to the pre-B cell, an immature B cell, and the prothymocyte, an immature T cell. B cells and T cells undergo differentiation in the bone marrow and thymus, respectively [6]. Because of the lack of self-

replenishing or multipotent stem cells in the thymus, continuous input of prothymocytes is needed for further differentiation. Once the prothymocyte enters the thymus, rearrangement of the TCR gene occurs. TCR diversity is achieved by the random use of variable (V), diversity (D), and joining (J) segments. Although the function of γ/δ -T cells is unclear, these cells, similar to cytotoxic T cells, are known to lyse target cells by the perforin and granzyme system and to secrete interferon γ (IFN- γ) and TNF- α on activation. In allergic airway disease, T cells with γ - and δ -chains may have a regulatory function [47,48].

After successful rearrangement of the TCR, thymocytes express CD4 and CD8 on their surface (CD4⁺CD8⁺). During development, the thymocyte undergoes positive and negative selection. If a thymocyte weakly recognizes a self-peptide MHC complex on the epithelial cells of the thymic cortex, it undergoes positive selection. If thymocytes are not positively selected, they die by apoptosis by a default pathway. The positively selected thymocytes enter the medulla and become single positive, expressing either CD4 or CD8. If the TCR has high affinity to the self-MHC peptide complex, apoptosis occurs by negative selection. The surviving thymocytes enter the circulation as naïve α/β -T cells and usually migrate to the peripheral lymphoid organs. These mechanisms of selection help prevent autoimmunity.

Membrane CD3, a component of the TCR signaling cascade, is present after week 10 of gestation [49]. By 14 weeks of gestation, the three major thymocyte subsets (as defined by surface expression of CD4, CD8, and T-cell receptor complex molecules) are found in the fetal liver and spleen, and CD4⁺ T cells are detectable in the follicles of the primary lymph node [50]. The percentage and absolute number of CD4⁺ T cells are higher in neonates than are those in adults and decline after birth. The percentage and absolute number of CD8⁺ T cells are lower in neonates than are those in adults and steadily increase through the first 5 years of life. The ratio of CD4⁺ and CD8⁺ declines from about 4:1 to 5.1:1 at 28 weeks of gestation to 2.5:1 to 3.1:1 at term [51].

After naïve T cells contact antigens presented by APCs, a small number of cells persist as memory T cells [52]. Human T cells express different surface markers according to their memory status: CD45RA for naïve cells and CD45RO for memory cells. Surface markers such as CD29 and CD58 are associated with the naïve and memory classifications. In human cord blood, CD45RA is expressed on 80% to 90% of the T cells, compared with 40% to 60% in adult cells [53]. Memory T cells infrequently are found in the newborn blood and spleen, but comprise approximately half of the T cells in adult tissues. These cells express high levels of the IL-2R α chain and proliferate in response to the administration of IL-2 [54].

T-cell activation by way of the costimulatory pathway

The interaction of the TCR with an antigenic peptide that is bound to the MHC leads to phosphorylation of the cytoplasmic domain of the CD3 complex. Naïve T cells need additional costimulatory signals to induce T-cell proliferation and cytokine secretion. The most fully characterized costimulatory interactions

are between CD80 and CD86 on APCs and between CD28 and cytotoxic T lymphocyte-associated molecule-4 (CTLA-4) on T cells. CD28 enhances T-cell proliferation, IL-2 synthesis, and differentiation. CTLA-4 initially was proposed to provide a costimulatory signal in conjunction with TCR signaling [55]; however, CTLA-4 functions to actively inhibit T-cell responses. The medullary dendritic cells in the fetus express high levels of CD80 compared with dendritic cells in the adult [56]; however, the role of these high levels of expression in the fetus has not been determined. Although experimental costimulation with monoclonal antibody to CD28 enhances IL-2 production in the fetus, IL-2 levels remain lower than in adults, suggesting further activation requirements in the neonate [57].

An additional costimulatory pathway, CD40-CD40 ligand (CD40L), is as mature in neonates as it is in adults [58]. This pathway is required for production of IL-12 by dendritic cells after antigen presentation to naïve T cells. In murine CD4⁺ T cells, blockade of CD40L with monoclonal antibody inhibits IFN- γ production, whereas secretion of IL-4 is increased [59]. In humans, activated CD4⁺ T cells induce production of IL-12 by monocytes by way of the receptor interaction between T cell-associated CD40L and CD40 on monocytes [60]. Neonatal T cells express less CD40L on their surface and RNA than do adult cells, whereas primed and restimulated neonatal T cells express comparable levels of CD40L in vitro [61]. Durandy and co-workers found that mitogenic stimulation induces CD40L in adult PBMCs and in cord blood mononuclear cells (CBMC) from fetuses between 19 and 28 weeks of gestation. CD40L expression was not inducible in term infants despite longer incubation periods with higher doses of mitogen [62].

Another costimulatory pathway is that involving OX40 (a member of the TNF-receptor family, found on T cells) and OX40L (expressed primarily on APCs and activated T cells). In cells derived from umbilical blood, the OX40–OX40L interaction promotes the development of naïve T cells to T helper type 2 (T_H2) effector cells [63]. These findings have not been compared in experiments with cells derived from adults; however, the T_H2-promoting involvement of OX40 was confirmed in a murine model of leishmania infection and in in vitro studies [64,65].

Proliferative response in T cells

T-cell development. In an analysis of human fetuses and premature infants between 15 and 34 weeks of gestation, Jones et al reported a strong correlation between gestational age and mitogen-inducible, lymphocyte proliferative response in PBMCs. The stimulation index (ratio of stimulated cells to unstimulated cells, ³H thymidine incorporation) increased for phytohaemagglutinin, cat fur, birch pollen, and β -lactoglobulin, which correlated with a gestational age greater than 22 weeks [66]. Neonatal T cells from term infants proliferate in response to mitogens and bacterial superantigens [67–69] at levels similar to those in adults. Cord blood T lymphocytes have a diminished

response to monoclonal antibodies to CD2, an adhesion molecule [70], or CD3 [71]. The significantly lower lymphocyte proliferative responses of neonatal T cells might be caused by the large proportion of naïve T cells in CBMCs. Purified neonatal CD4⁺ CD45RA T cells have been reported to proliferate substantially more in response to IL-4 than do adult cells, suggesting that neonatal naïve T cells have a higher potential to become T_H2 cells than do naïve adult T cells [72].

Allergen-specific proliferation in cord blood

Whether an allergen-specific lymphocyte proliferative response is caused by priming in utero is controversial. Several authors have suggested that in utero priming is the reason why some allergens, particularly food-derived allergens, are more likely than others to induce an allergen-specific response in CBMCs [73–78]. The concept of in utero priming suggests that aeroallergens may cross the placenta. Data on this subject have been conflicting. Newborns transiently develop a lymphocyte proliferative response to egg-white albumin (OVA), but this response disappears in adults. With aeroallergens, the reverse effect is found (adults respond, whereas neonates do not) [79]. The dose of the allergen may affect in utero sensitization, as intake of milk protein by a pregnant woman is estimated as 100 g/d, and the intake of pollen or house dust mite allergens is an estimated 10 ng/d [80]. Szepfalusi and colleagues reported a 7-month-old child with a positive lymphocyte proliferative response to birch pollen who had never experienced a birch pollen season. The presence of specific immunoglobulin G (IgG), IgM, and IgA supports a possible exposure in utero [81]. Investigation of the relationship between exposure to house dust mites at home and CBMC-specific responses revealed no association between exposure, allergen-specific proliferation, and development of atopic diseases [82]. Smillie and co-workers investigated the relationship between lymphocyte proliferative responses at birth and at 1 year of age and the exposure of the mother and infant to dust mites. They found no relationship between levels of dust mite (Der p 1) in the mother's bed and CBMC proliferative responses. CBMC proliferative response did not predict the 1-year PBMC response. The proportion of positive proliferative responses was increased significantly with increasing Der p 1 exposure at 1 year of life. Categorizing neonates into those at high and low risk for atopy according to the presence of parental atopy revealed a significant difference in mitogen-induced lymphocyte proliferation: High-risk neonates had a lower proliferative response than did low-risk neonates. In contrast to the report by Prescott et al [78], a CBMC proliferative response to tetanus toxoid was detected by Smillie and colleagues [83]. One possible explanation for this response is that the concentration of tetanus toxoid used in the Prescott study was 60 times lower than the optimum concentration established by Smillie et al. Because maternal exposure to tetanus toxoid is unlikely during pregnancy, the proliferative response is more likely caused by a nonspecific response than a maternal–fetal transfer of the peptide.

Van Duren-Schmidt and co-workers also examined aeroallergens using the lymphocyte proliferative response of umbilical cord blood (UCB) as a marker for exposure during pregnancy. Most proliferative responses to birch pollen and timothy grass occurred in the UCB samples in which the pollen season (ie, antigen contact) took place in the first 6 months of pregnancy. Proliferative responses were not necessarily dependent on maternal exposure, which argues against the hypothesis that priming occurs in utero by way of allergen transfer [76]. In an extended study based on the same cohort that examined samples obtained from cordocentesis, the allergen-specific T-cell reactivity to inhalant allergens was as common in preterm as in term infants [84].

As demonstrated earlier, the data on in utero priming is indeterminate. It is possible that in utero allergen exposure and subsequent sensitivity may occur and may be dependent on gestational age.

T_H1 and T_H2 cytokine pattern in cord blood

T helper cells are categorized as T_H1 and T_H2 cells on the basis of their pattern of cytokine secretion (eg, T_H1 secretes IFN- γ , IL-2, TNF- β ; T_H2 secretes IL-4, IL-5, IL-13). This classification scheme initially was described in a murine model. Different diseases are associated with a predominance of T_H1 or T_H2 responses (eg, allergic asthma and T_H2). This concept is now under evaluation, and recent investigations have questioned the relationship between atopic diseases and the T_H1 and T_H2 paradigm. Detection of T_H2 cytokines, such as IL-4 and IL-5, at the level of protein in cord blood has presented a challenge, often because IL-4 and IL-5 proteins are below the detectable level of the standard, commercially available cytokine assays. Usually, mRNA analysis is performed in conjunction with analysis of other cytokines at the protein level [85, 86]. After neonatal CD4⁺ T cells are stimulated with anti-CD3 and anti-CD80, they release high levels of T_H1 cytokines (IFN- γ , IL-2, TNF- α , TNF- β) and low levels of T_H2 cytokines (IL-4, IL-13). A deficiency in IFN- γ production in individuals who are at risk for atopy or for early signs of atopic disease results in the absence of the T_H1 profile, thereby skewing toward a predominantly T_H2 response [75,86–89]. In contrast, Williams and colleagues reported that CBMCs from term infants with atopic symptoms by 3 years of age and with a parental history of atopy produced significantly lower concentrations of mitogen-induced IL-13 than did the CBMCs of infants with no parental history of atopy [90]. An ongoing prospective study by Hagendorens et al evaluating the T-cell subpopulations and intracellular cytokine production did not find a difference between the mitogen-induced cytokine production of CBMCs of infants with a parental history of atopy and the cytokine production of infants with no history of parental atopy. To examine the association between clinical symptoms of atopic diseases and immune function, these results will be reevaluated when the atopic status of the children can be assessed [91].

Warner and co-workers investigated allergen-specific cytokine production and found a low IFN- γ production in response to stimulation with β -lactoglobulin in PBMCs from subjects with atopic eczema at 1 year of age and a trend for similar

responses to OVA in a cohort of 34 newborns at risk for atopy [75]. Extending the postnatal observation time, Kondo and colleagues found that the IFN- γ response of CBMCs to food allergen is associated with atopic disease by 6 years of age [92]. As mentioned earlier, allergen-induced cytokine production from CBMCs is skewed toward T_H2 cytokines as evidenced by measurement of mRNA for IL-4, IL-5, IL-9, IL-10, and IL-13. IL-10 and IL-13 also were readily measurable at the protein level [86]. A subsequent report by the same investigators indicated that T_H2 responses in CBMCs were higher than were those in normal newborns but showed a rapid down-regulation during the first year of life [93].

Miller et al studied 167 pregnant, African-American, or Dominican women for the correlations between prenatal maternal allergen exposure and newborn cord and between maternal blood proliferation and cytokine production. CBMC proliferation to antigen occurred even in the absence of antigen-induced PBMC proliferation in the mother. Antigen-induced IL-5 production in cord blood correlated with the proliferative response to dust mite extracts [94]. Maternal blood was drawn 1 day after delivery, however, and may not reflect the response of PBMCs in nonpregnant women, because pregnancy can modify the immune response (see later in text).

Kopp and colleagues examined the difference between the maternal PBMC and fetal CBMC proliferative and cytokine responses to β -lactoglobulin. Higher IL-13 protein production was observed in CBMCs from neonates with sensitized mothers (defined by specific IgE) compared with that in those with nonsensitized mothers. The production of IL-5 and IL-10 did not differ significantly within the sensitized and nonsensitized neonates and mothers. Sensitized mothers had significantly lower IFN- γ production than did nonsensitized mothers [95].

Several studies investigated the association between bacille Calmette-Guerin (BCG) vaccination and atopic diseases, including asthma, in response to the suggestion that BCG vaccination induces T_H1 cytokine predominance [96, 97]. Subsequent studies did not provide a definitive answer as to the effect of BCG on the T_H1 response. A retrospective study by Shirakawa found an inverse association between tuberculin responses and atopic disorders in Japanese school-children, which suggests a protective effect against atopy [98]. A retrospective cohort study in Swedish children with atopic heredity did not find any effect of BCG vaccination on the development of atopic diseases [99]. No association between BCG vaccination and atopy and asthma was noted in a Chinese cohort [100]. In a German cohort, a trend toward a protective effect of BCG vaccination was found initially but disappeared with increasing age of the children [101]. In contrast, in African children, BCG vaccination seems to prevent atopy [102]. These contrasting results are most likely caused by differences in antigen exposure, environment, and genetic background.

Inflammation and transcription factors

Transcription factors regulate immune genes critical for cytokine production. Some transcription factors (eg, NF- κ B) are more involved in the inflammatory

response and are found in many cell types. Other transcription factors are highly specific to certain cell subtypes (eg, signal transducers of activated T cells (STAT) 4, 6, GATA-3, nuclear factor of activated T cells [NF-ATCs]). In murine model of allergic inflammation, NF- κ B is critical for inflammation and AHR. Mice deficient in the NF- κ B subunit c-rel did not develop increases in pulmonary inflammation, bronchoalveolar lavage eosinophilia, and total serum IgE and did not exhibit AHR [103]. Mice deficient in the NF- κ B subunit p50 were unable to generate IL-5, eotaxin, macrophage inflammatory protein 1 α (MIP-1 α), and MIP-1 β , chemokines that have been implicated in recruitment of T cells to sites of inflammation [104]. The inhibition of the NF- κ B pathway in transgenic mice that overexpress the inhibitor I κ B leads to severe impairment of T_H2 type cell responses and a predominance of T_H2 cell-dependent responses [105]. A report by Lawrence and colleagues demonstrated that the role of NF- κ B was not limited to induction of proinflammatory genes and that NF- κ B also activates anti-inflammatory genes and apoptosis, depending on the stage of inflammatory resolution [106].

Whether NF- κ B activity differs in neonates and adults has been the subject of recent studies. The addition of antibodies to CD2 and CD28 to neonatal and adult T cells resulted in increased NF- κ B DNA binding in neonates (n = 3) and adults. The neonatal levels of NF- κ B were lower than that of adults, possibly implying decreased NF- κ B induction. This finding suggests that T cells in the neonate have additional activation requirements compared with adult T cells [57]. The author has found that NF- κ B is regulated differentially on mitogen stimulation of CBMCs (C.H. Schroeter, unpublished data 2002). Whether NF- κ B activity that is detected in the neonate is associated with different clinical outcomes is under investigation.

Macaubas and colleagues investigated mRNA expression of GATA-3, a T_H2-specific transcription factor, in human T_H2-polarized CBMCs. Their findings, consistent with those of murine studies, showed increased GATA-3 during T_H2 development but decreased levels during T_H1 development. Whereas adult atopic subjects showed increased levels of GATA-3 mRNA after allergen stimulation, nonatopic subjects showed decreased levels [107]. No studies have examined allergen-induced regulation of GATA-3 in neonatal cells. Kadereit et al, whose study focused on nuclear factor of activated T cells-1 (NFAT1), showed that NFAT1 had no constitutive expression and was only partially up-regulated after prolonged stimulation, as compared with adult T cells [108]. These studies support the concept that differential regulation of transcription factors may occur in neonates versus adults.

Maternal immune system during pregnancy and maternal–fetal interactions

Changes of the maternal immune system during pregnancy

To accept the fetus as an allograft, the maternal immune system must undergo several changes. Although the immunologic basis for this acceptance has

not been illuminated completely, investigations contribute to the understanding of immunologic changes during pregnancy. This section briefly addresses changes in T-cell proliferation and cytokine production during pregnancy. For a more detailed review, readers are referred to the study by Mellor and Munn [109].

Pregnancy is notable for hormonal fluctuation. Prabhalha and colleagues showed, in a murine model, that hormone levels influence antigen presentation in the mucosa during reproductive cycles [110]. In transgenic pregnant mice, maternal T cells have a transient tolerance for paternal alloantigens, which is limited only to pregnancy [111]. Human pregnancy is considered primarily a maternal T_H2 state [112–115]. Because IFN- γ is cytotoxic to the placenta, a T_H1-predominant response may lead to placental detachment and fetal loss [112]. Consequently, down-regulation of any T_H1 state may be necessary for a successful pregnancy. The fact that the placenta cytotrophoblast itself produces high levels of IL-4 [116], IL-10 [117,118], and other mediators that effect T_H1 and T_H2 differentiation (eg, prostaglandin E₂ [PGE₂] [119]) supports this hypothesis.

Murine models have shown that cytokine imbalance that is associated with an impaired activation of macrophages and NK cells has a major role in the survival of the fetus. One review classified cytokines as beneficial (IL-1, IL-3, IL-4, IL-6, IL-10, transforming growth factor- β [TGF- β], colony stimulating factor-1 [CSF-1], granulocyte-monocyte colony-stimulating factor, IFN- τ) and deleterious (TNF- α , IFN- γ , IL-2) in animal models of pregnancy [120]. The injection of IL-2, a T_H1-polarizing cytokine, leads to increased rates of fetal loss in a pregnant murine model of human habitual abortion [121]. Other studies have shown that high levels of constitutive IFN- γ production by placental intervillous blood mononuclear cells effectively can control *Plasmodium falciparum* infection in the placenta on exposure [122]. Mycobacterial antigens induce a predominantly IFN- γ response [123]. These data suggest that IFN- γ may have a beneficial role in the avoidance of infection in the fetus. Piccinni investigated the effect of progesterone on cytokine production by antigen-specific CD4⁺ T cells and showed that a T_H2 cytokine profile in response to antigens is induced in the presence of progesterone [124]. This effect might be caused by a direct inhibition of the K⁺ channel, which results in inhibition of Ca²⁺ signaling and NF-AT-driven gene expression [125]. PGE₂ inhibits the proliferative response to mitogens, the production of IL-2 and IFN- γ by PBMCs and splenocytes, and the production of IL-4 and IL-10 [126]. One origin of PGE₂ is the decidua, which promotes T_H2 cytokine production [127].

Placental barrier and its immune-regulating functions

The placental barrier is composed of villous structures, such as the syncytiotrophoblast and cytotrophoblast, their basal membranes, the stroma, the endothelium, and the basal membranes of villous capillaries. The fetal part of the placenta is composed of fetal tissues, such as the trophoblast, amnion, mesenchyme, and fetal

blood vessels. On the cellular level, the placenta contains lymphocytes and an abundance of macrophages, which have a role in cytokine production and fixation of maternal antibodies [128]. The trophoblast is also in contact with the decidual tissue. In the decidual tissue, approximately 10% of the cells are maternal lymphocytes, and 20% are maternal macrophages, which interact with the decidual cells. The secretory component of the placenta (mucosa) includes IgA and IgM. The placenta forms a physical barrier to cellular exchange that may lead to spontaneous abortion. The placenta provides passive immunity and nutrients to the fetus and detoxifies metabolic byproducts. This barrier is not completely impermeable and may leak. Maternal T cells that are primed against fetal alloantigens have been found [111], as have maternal cells in the fetus and fetal cells in the mother.

Maternal–fetal interface

Fetal-to-maternal transfer

Almost a century ago, Schmorl and colleagues found trophoblasts in the maternal lung after eclampsia [129]. Evidence suggests that eclampsia is associated with an increased rate of cell traffic [130]. Fetal, nucleated red blood cells can be detected in maternal circulation [131]; however, despite the use of cell enrichment of trophoblasts and nucleated erythrocytes [132], not all authors have been successful in detecting fetal cells in the maternal blood [133,134], suggesting that maternal–fetal cell traffic may not occur in every pregnancy. Fetal cells are detectable during pregnancy and directly after delivery [135], but one report noted the presence of progenitor cells 27 years after the pregnancy [136].

Lo and co-workers reported the presence of fetal cells in the maternal circulation in 26 of 51 samples. Kuo et al looked for fetal cells in the maternal circulation during different gestational stages and found that the number of nucleated red blood cells increased steadily from 5.3 cells/10 mL of maternal blood during early gestation (5–10 weeks) to 98.2 cells/10 mL near term. The number of cells with a male karyotype identified by polymerase chain reaction (PCR) increased from 6 during early gestation to 31 during the second trimester and decreased slightly to 27 near term. Both cell types disappeared at 3 months after delivery [137].

Maternal-to-fetal transfer

Previous studies have suggested that maternal cellular contamination of CBMCs used for bone marrow reconstitution in an adult may lead to rejection of the transplant. For this reason, the question of maternal contamination of CBMCs must be addressed. Two early reports found leukocytes and radiolabeled maternal erythrocytes in the fetal circulation [138,139]. Wernet and colleagues investigated 16 cord blood samples and looked for genotype and phenotype by fluorescent in situ hybridization (FISH) combined with confocal laser-scanning

microscopy with a sensitivity of 0.1%. Maternal cells were detected in one sample, without a phenotypic description [140].

Using PCR methods to detect minisatellite sequences, Socié and co-workers investigated 47 cord blood samples and found maternal-specific alleles in one sample [141]. In a follow-up study that examined different gestational ages, maternal cells were found between 24 and 35 weeks of gestation in eight of nine samples in estimated amounts of 0.1% to 0.01% [142].

Hall et al detected maternal cells by FISH to the X and Y chromosome in 10 of 49 cord blood samples. The levels ranged from 0.04% to 1.0% of maternal cells after counting 1000 nuclei. Fractionation of the CD8⁺ and CD34⁺ population revealed maternal cells in 5 of 39 CD8⁺ fractions and in 1 of 27 CD34⁺ fractions [143]. Lo and colleagues reported that 16 of 38 fetal UCB samples were positive for maternal cells. Fetal cells were detected in 26 of 51 maternal peripheral blood samples [144]. Blood was available from both mother and infant in 28 cases. No correlation was found between the presence of maternal cells in the infant's cord blood and the presence of CBMCs in the mother's peripheral blood. These data suggest that cell transfer is likely to be more than a passive transfer phenomenon at the time of delivery. Additional studies by the same group examined fetus-to-mother transfer of nucleated cells and plasma DNA in pregnant women who were carrying male babies. Maternal DNA was detected in the cellular fraction of UCB in 12 of 50 samples. Fetal-to-mother transfer of nucleated cells was detected in 13 of 50 samples [145]. A case report by Paoloni-Gaicobino of materno–fetal transfusion described a normal male fetus after hypoxia-induced death caused by a four-fold twisting of the umbilical cord in utero at 35 weeks of gestation. The maternal cells were estimated to comprise 5% to 10% of the nucleated fetal blood cells [146].

To determine whether maternal cellular contamination was responsible for the allergen-induced response observed in CBMCs, Prescott derived T-cell clones, which were specific for OVA or house dust mite allergen from lymphoblasts from CBMCs. DNA was extracted from the clones and compared with DNA extracted from peripheral blood mononuclear cells from the mother and the 6-month-old CBMC donor infant. Microsatellite genotyping at two polymorphic gene loci illustrated the fetal origin of the clones [78]. Clerici selected three male samples ($n = 24$) and performed cytogenetic analysis of metaphases by analyzing 450 nuclei for X and Y chromosomes. No female karyotype was found, indicating that no contamination occurred [69].

These studies indicate that there is bidirectional cell traffic between the mother and fetus. Although maternal cellular contamination of fetal cord blood seems to be minimal, the impact of the maternal-to-fetal exchange remains to be determined.

Immune-mediator traffic

Although there is some degree of maternal–fetal cellular traffic, the maternal–fetal interface presents a unique, highly selective barrier to immune-system mediators. The only immunoglobulin known to cross the placenta in significant

amounts is IgG. Small amounts of IgG cross from the maternal blood to the fetus as early as week 12 of gestation [147]. The amount of IgG in fetal serum increases significantly between week 22 and 26 [148]. A retrospective review of 96 publications found that concentrations of fetal IgG are low at the beginning of pregnancy, increase to more than 2.5 g/L after 25 weeks, and reach 5 g/L at week 30. By week 35, concentrations of fetal IgG exceed concentrations of maternal IgG [149].

Transport of immunoglobulin

Maternal IgG is transported by way of a specific mechanism. The uterine artery carries maternal blood to the chorionic villous. The maternal endothelium is in contact with the syncytiotrophoblast, which is the outermost layer of the villi. The villi are supplied by fetal umbilical arteries, which are connected by capillary networks to the umbilical veins. IgG enters the syncytiotrophoblast by receptor-mediated endocytosis and is transported in vesicles across the fetal endothelium [150]. Fc receptors for IgG have been found on the syncytiotrophoblast [150], Hofbauer cells, and fetal endothelium [151]. Known Fc receptors in the placenta are Fc γ RI (CD64), Fc γ RII (CD32), Fc γ RIII (CD16), and the neonatal Fc receptor, FcRn [152].

Several studies have shown permeability of the placenta to multiple antigens and specific fetal immune responses to these antigens. Gill and co-workers immunized 42 pregnant women with tetanus toxoid during the fifth and eighth months of pregnancy and compared the immune response of the offspring with that of children of nonimmunized mothers. The babies of immunized mothers had specific IgM in their cord blood that was not present in the controls. Babies of immunized mothers responded earlier (by 7 months of age) than did control babies to diphtheria-pertussis-tetanus vaccination. The lymphocytes of the babies from immunized mothers remained sensitized to tetanus toxoid 13 months after birth, whereas those of the control babies did not [153]. These data support the notion that transplacental immunization of the fetus may occur under certain circumstances.

The maternal–fetal interface provides a means for passive immunity by way of maternal IgG and antigen-mediated sensitization, priming the fetal immune system for life outside the womb.

Interaction between the innate and adaptive immune systems

As outlined earlier, the innate immune system is the body's most ancient form of defense and exists in invertebrates and vertebrates. The adaptive immune system exists only in vertebrates and evolved after innate immunity. It is questionable why the more newly evolved and sophisticated system of defense does not cause the innate immune system to become obsolete. It is evident that the function of the adaptive immune system remains dependent on aspects of the innate immune system and that they are linked inextricably.

Inflammatory mediators elicited by innate immunity

The innate immune system depends on the interaction between the microbial, pathogen-associated molecular patterns and the pattern recognition receptors found on effector cells, such as APCs. This interaction, discussed earlier, leads to the release of inflammatory mediators, such as IL-1, TNF- α , IL-6, type I IFNs, and chemokines [154]. Mediators that affect a T_H1 (IL-12, IFN- γ) versus a T_H2 response (IL-4, IL-5, IL-10) are released [154,155]. These mediators control, influence, or induce aspects of adaptive immunity, which impact susceptibility to develop allergic diseases.

Costimulation

Targets of the innate immune system, such as bacterial DNA, viruses, LPS, and mannans, have been shown to induce expression of costimulatory molecules on the cell surface, thereby influencing adaptive immunity [156,157]. Unmethylated cytidine-guanosine dinucleotide (CpG) bacterial DNA stimulates dendritic cells to secrete T_H1-like cytokines (IL-12, IL-18) [156]. CpG DNA induces expression of costimulatory molecules, such as intercellular adhesion molecule 1 (CD54), CD86, and CD40, on human peripheral blood dendritic cells [158]. LPS also induces B cells to provide costimulatory signals to naïve and memory CD4 T cells [157].

Complement

Mannan-binding protein and other PAMPs activate the complement cascade. The C3 cleavage product C3d tags the antigen that has been recognized by the innate immune system. The plasma membrane of B cells contains a coreceptor made up of CD19, a component of the acquired immune system; CD21, a C3d protein receptor; and the target of the anti-proliferative antibody-1 (TAPA-1) [159]. The tagged antigen binds the B-cell receptor and the B-cell coreceptor by way of CD21. This step induces phosphorylation of the cytoplasmic tail of CD19. Coligation of the B-cell receptor with its CD19/CD21/TAPA-1 coreceptor increases signaling 1000 to 10,000 fold [6]. These receptors are crucial for humoral immunity, because mice deficient in CD21 or CD19 have been shown to have an impaired humoral response to T-cell-dependent antigens [160–162]. Complement seems to be important in the response to LPS, because C3- and C4-deficient mice are more sensitive to endotoxin than are wild-type controls [163].

Self versus non-self

The innate immune system has the capacity to determine self versus non-self and, in its lack of response to self, exerts control over adaptive immunity [154]. Self-antigens lack the PAMPs that are recognized by the innate system. Self-antigens do not induce the costimulation that is necessary for activation of T cells specific for self-antigens. Without costimulation, these self-specific T cells

become tolerant or anergic. The innate immune system directly controls the activation of adaptive immune responses.

Summary

The advent of molecular tools, transgenic models, and genomic approaches has resulted in an explosive increase in the understanding of the immune system. This article considers how advances in the understanding of innate and adaptive immunity have crafted the knowledge of the fetal immune system, this system's interaction with the maternal environment, and the impact on the development of allergic diseases. Future examination of these pathways, their intersection with environmental exposure, and the interactions between the innate and adaptive immune systems may provide attractive therapeutic approaches for the treatment and prevention of allergic diseases.

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