

DEVELOPMENTAL IMMUNOLOGY OF THE NEWBORN

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The increased susceptibility of newborns, especially those born prematurely, to serious infections has been well documented.^{40, 48, 62} Despite advances in antimicrobial therapy and neonatal intensive care, neonatal infections continue to be an important determinant of childhood morbidity and mortality in both developed and Third World countries.⁹ The incidence of neonatal sepsis has remained unchanged and ranges from 1 to 10 per 1000 live births with an even higher incidence of 21 per 1000 live births among the developing countries where access to sophisticated "modern" technology is limited.^{23, 40, 141} Concurrent meningitis, associated with a high risk of neurologic morbidity, occurs in about one fourth of cases.⁴⁰ In the preantibiotic era, neonatal sepsis was almost always fatal. Mortality has decreased significantly after the introduction of antimicrobial agents and technologic advances in neonatal management. In the past 15 years, the case-fatality rate for noninfectious neonatal illnesses has continued to decline, whereas that for infectious diseases has reached a plateau of approximately 15% for late-onset sepsis (sepsis that occurs between 4 and 30 days of life), and 15% to 50% for early-onset sepsis (sepsis that occurs in the first 4 days of life).⁶⁴ The impact of neonatal infections as a significant cause of neonatal death globally is more evident in developing countries, as recently reviewed by Stoll.¹⁴¹ Neonatal infections are responsible for between 1.5 and 2 million neonatal deaths per year, or between 4000 and 5000 deaths per day in the less developed countries of the world.¹⁴¹ The incidence of neonatal sepsis is even higher and inversely proportional to the gestational age and birth weight. In Israeli, Finnish, and American studies, very low birth weight infants (VLBW; weighing <1500

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g) had a sepsis rate almost twice as high as that of infants weighing 1500 to 2000 g and almost eight times that of infants weighing 2000 g or more.⁸⁰ The factors responsible for the neonate's predilection to infection are multifactorial. Obstetric factors, monitoring devices, and therapeutic procedures in the neonatal intensive care unit that interrupt the natural skin and mucosal barriers may predispose the neonate to infections.¹⁵⁶ Deficiencies in the various arms of the immune system clearly play major roles in this susceptibility to infections.

The immune system is composed of the innate or nonspecific host defenses, including natural epithelial barriers, cellular elements (e.g., eosinophils, basophils, neutrophils, platelets, and monocytes), and serum humoral components (e.g., kinins, coagulation factors, and complement proteins). The adaptive or specific host defenses include cell-mediated and antibody immune responses. This article reviews the current understanding and provides an update of the developmental aspects of the neonate's specific host defense systems. The reader is referred to recent reviews for the development of the innate or nonspecific host defense system.^{16, 65}

ONTOGENY OF T CELLS

Thymic Development and Early T-Cell or Thymocyte Development

The thymus arises from the endodermal and ectodermal layers of the embryonic structures known as the third pharyngeal pouch and third branchial cleft, respectively. The ectodermal layer gives rise to the thymic cortex, whereas the thymic medulla originates from the endodermal layer. In humans, the first evidence of thymus formation occurs around the sixth to seventh week of embryonic life.^{140, 142} By 8 weeks of gestational age, lymphoid cells with the prothymocyte phenotype (e.g., CD7+ but lacking other T-cell markers) migrate from the yolk sac and fetal liver and later from the bone marrow to colonize the fetal thymus.⁶¹ It is still unclear how these precursor cells are committed to differentiate along the T-cell lineage prior to entering the thymus and what attracts them to the thymic tissue. It has been postulated that these precursors may express certain surface markers, as yet unidentified, that selectively bind to their corresponding ligands on the thymic vascular endothelial cells. Once in the thymus, the prothymocytes interact with the stroma in the thymus and are triggered to undergo intense proliferation, differentiation, and expression of the first T cell-specific surface molecules (e.g., CD2 and later CD4 and CD8). A clear delineation of the thymic cortical and medullary regions occurs at 12 weeks of gestation⁶⁹ and shortly thereafter Hassall's corpuscles appear.¹⁴³ The most immature thymocytes are found in the subcapsular cortical region, whereas the more mature thymocytes reside in the deeper layers of the thymus. Prothymocytes do not express CD3, the T-cell receptor (TCR), CD4, or CD8 and are often referred to as triple-negative thymocytes. Prothymocytes migrate into the subcapsular region where they undergo vigorous proliferation and give rise to progeny that continue to divide and rearrange their TCR genes. These progeny now express both CD4 and CD8 and are called double-positive thymocytes. They migrate to the deeper layers of the thymus cortex, where positive selection for self-major histocompatibility complex (MHC) restriction occurs, a process known to be governed mainly by the thymic cortical epithelial cells.⁷³ The cells that fail positive selection never progress further than the double-positive stage and eventually die by apoptosis within 3 to 4 days after their last cell division. It

has been estimated that more than 95% or roughly 5×10^7 thymocytes die each day during this stage, leaving only 5% of the thymocytes to undergo further differentiation. Negative selection occurs next and is mediated by the bone marrow-derived antigen-presenting cells (APC) (e.g., dendritic cells and macrophages), which eliminates autoreactive cells either by clonal deletion or clonal anergy.¹⁰ By the time the thymocytes arrive into the medulla, most of the cells are considered mature and express either CD4 or CD8. These single-positive T cells migrate from the thymus to the peripheral lymphoid organs. The first evidence of the detection of CD4+ and CD8+ T cells in the fetal liver and spleen occurs by 14 weeks of gestation,⁴ which indicates that mature T cells migrate from the thymus to the periphery at this point in gestation.

Progress has been made in our understanding of intrathymic trafficking. Two thymus-derived chemokines have demonstrated recently that they play an important role in the migration of thymocytes into the deeper layers of the thymus as they differentiate into mature T cells. This differentiation-dependent thymocyte migration may be important for T lymphopoiesis and is probably regulated by thymic chemoattractants. Two chemokines, SDF-1, a CXC chemokine, and CK β -11/MIP-3 β /ELC, a CC chemokine, are highly expressed in the thymus. The chromosomal location in humans for the SDF-1 and CK β -11/MIP-3 β /ELC genes is 10 and 9, respectively. As observed by Kim et al⁷⁹ in a mouse model, SDF-1 is preferentially chemotactic for immature thymocytes, including those that are triple-negative and double-positive. CK β -11/MIP-3 β /ELC shows a strong chemotactic preference for more mature thymocytes (single positive CD4+ and CD8+ T cells). Moreover, the receptor for SDF-1 (e.g., CXCR4) is higher on double-negative, double-positive, and on some CD8 single-positive thymocytes. In contrast, the receptors for CK β -11/MIP-3 β /ELC (e.g. CCR7) are higher on CD4 single-positive thymocytes.⁷⁹ More information is still needed to understand the homing of precursor thymocytes and the intrathymic trafficking of T-cell progenitors.

The thymus is the largest lymphoid tissue relative to body size during fetal life. At birth the thymus is two thirds of its mature weight and reaches its peak mass at around 10 years of age. Subsequently, the thymus continues to involute and is replaced by adipose tissue.³⁵

Generation of T-Cell Receptor Diversity

Diversity in the $\alpha\beta$ and $\gamma\delta$ T-cell receptors and the immunoglobulin receptor is generated from a complex sequence of DNA recombinatorial events (Fig. 1). These events occur on chromosome 7 for the β and γ chains of the T-cell receptor and on chromosome 14 for the α and δ chains of the T-cell receptor. The mechanisms by which these receptor molecules are generated have been reviewed elsewhere.^{49, 86} Receptor diversity is generated by the assembly of several gene segments using unique recombinase enzymes that splice the DNA segments. The TCR is assembled from germ line-encoded gene segments. The TCR V β genes are composed of variable (V), diversity (D) and joining (J) segments that are assembled through a process of DNA recombination (reviewed in 49, 86). The antigen-binding sites are located in the hypervariable regions that are generated from three hypervariable complementarity-determining regions (CDR). CDR2 comprises the periphery of the antigen-binding site, whereas CDR3 is the most extensive variable segment and is a key site for fine antigen recognition.¹²⁷ Mechanisms of receptor diversity allow the recognition of all possible antigens through a process of clonal expansion and a positive and

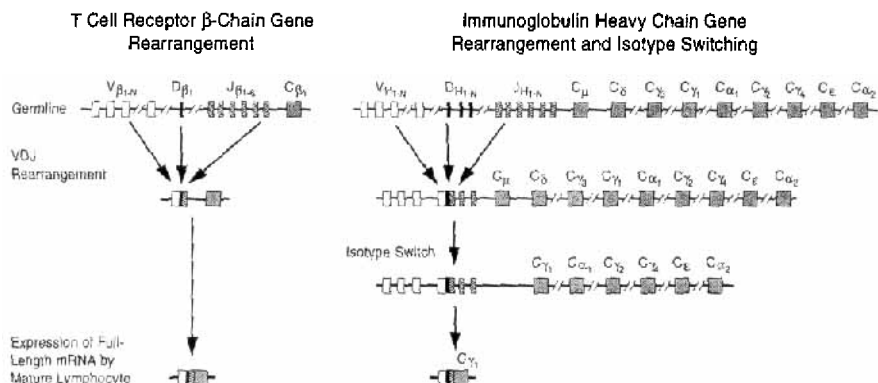


Figure 1. Rearrangement of the human T-cell receptor β -chain gene and the human immunoglobulin heavy chain gene. DNA rearrangements between the V (variable), D (diversity), and J (joining) region genes create a configuration for the antigen-binding site. The third hypervariable interval of the complementarity-determining regions (CDR3) is encoded by the joining process of the V(D)J gene segments, and lies at the center of the antigen-binding site. It is this region that determines the fine specificity (and affinity) for antigen binding and the diversity of the T-cell receptor and immunoglobulin repertoire. A similar process of DNA rearrangements occurs for the T-cell receptor- α , γ , and δ chain genes and the immunoglobulin light chain genes. (From Lewis DB: Cellular immunity of the human fetus and neonate. *Immunology and Allergy Clinics of North America* 18:295, 1998; with permission.)

negative selection process in the thymus for T cells²⁶ and in the bone marrow for B cells⁵⁷ to remove or anergize self-reactive clones.

By 15 weeks of gestation, human thymocytes express a complete set of TCR.⁵⁴ The terminal deoxynucleotidyl transferase (TdT) levels in fetal thymocytes are lower than in adult lymphocytes.¹²³ This enzyme mediates the addition of nucleotides at gene segment joints and is responsible for junctional diversity at the VD, DJ, or VJ segment joins. TdT⁺ cells cannot be detected at 10.5 weeks of gestation.¹⁹ Diminished TdT enzyme results in a shorter CDR3 region and a restricted TCR repertoire in fetal thymocytes. After 20 weeks of gestation, TdT expression increases along with an increase in N-region diversity.¹³ By 24 weeks of gestation, all TCR V β families are expressed, and the size of the CDR3 segment of the V β region is not substantially different from that in adults.¹²⁷ The capacity of term cord blood T cells to expand the TCR β repertoire after stimulation with potent stimulators (toxic shock syndrome toxin-1 [TSST-1], staphylococcal enterotoxin-1) is similar to that seen in adults.⁵²

By the middle of the second trimester, a complete set of functional TCR V β chains exist, but they have shorter CDR3 regions because of diminished TdT expression. Levels of this enzyme increase to those of adult levels by 20 weeks of gestation, resulting in an increase in N-region diversity and normal CDR3 region size heterogeneity.¹³ By completion of the third trimester of pregnancy, the T-cell V β repertoire is similar to that seen in adults.^{52, 127}

Development of $\gamma\delta$ T Lymphocytes

$\gamma\delta$ T cells represent a distinct functional subset of T cells. The majority of $\gamma\delta$ T cells lack surface expression of both CD4 and CD8 but have the complete

CD3 complex.¹⁴² There are controversies about whether these T cells originate from the same or different lineage of $\alpha\beta$ T cells and whether they go through an intrathymic selection process.⁷² The thymus may not be important for $\gamma\delta$ T-cell development because normal $\gamma\delta$ T cells can develop in athymic mice.¹⁴ The factors that influence $\gamma\delta$ T-cell differentiation and maturation are also unknown. In humans, $\gamma\delta$ T cells can be detected as early as 6 to 8 weeks of gestation in the fetal thymus and liver and are the first T cells to express TCR, shortly after they migrate into the thymus.⁶⁸ The $\gamma\delta$ TCR genes undergo rearrangement prior to the α/β TCR genes. Expression of the TCR- δ chain can be detected as early as 9.5 weeks of gestation.⁶⁰ At 16 weeks' gestation, $\gamma\delta$ T cells comprise about 10% of the peripheral blood T cells and then gradually decline to less than 3% of the circulating T cells at birth.¹¹⁰ Although the functions of these T cells are not well understood, some interesting features deserve mentioning. $\gamma\delta$ T cells are found predominately in the skin and mucosal surfaces, with less than 5% in the peripheral blood. $\gamma\delta$ T cells, similar to cytotoxic T lymphocytes (CTLs), can lyse target cells by the perforin/granzyme system and can secrete IFN- γ and tumor necrosis factor α (TNF- α) upon activation.⁶⁸ Neonatal $\gamma\delta$ T-cell cytotoxicity is significantly less than that in adults.¹⁰³ Another unique feature of these T cells is their restricted repertoire compared with that of $\alpha\beta$ T cells and B cells.¹³⁵

Fetal $\gamma\delta$ T cells have a more diverse repertoire but a more limited junctional diversity than adult $\gamma\delta$ T cells that have a more restricted repertoire. This diversity remains throughout the first year of life and gradually decreases to attain the adult repertoire for the TCR- δ chain by 14 years of age.⁶⁸

ONTOGENY OF PERIPHERAL T CELLS

Cell Number

By using monoclonal antibodies and ultrasound-guided fetal blood sampling for the prenatal diagnosis of congenital infections, the enumeration of lymphocytes and their phenotypes during fetal life can be evaluated.^{67, 118} The absolute number of mature T cells (CD3+) and the CD4+ and CD8+ subpopulations gradually increases from 19 weeks' gestation (Table 1). This progressive increase in the mature CD3+ T cells continues after birth, reaches a peak around 6 to 9 months of age, and declines to adult values at 6 to 7 years of age.^{44, 109, 143} The percentage and absolute numbers of CD4+ cells are higher in neonates compared with that in adults and decreases after birth. The CD8+ percentage and absolute numbers at birth are lower than those of adults and steadily increases through the first 5 years. These changes in CD4+ and CD8+ T-cell subpopulations are reflected in the CD4/CD8 ratio that is high during the perinatal period (as high as 4.9:1) and declines gradually to reach adult values of approximately 2:1 at 4 years of age.^{44, 45, 109} Thomas and Linch¹⁴⁷ compared the total T cells and their subsets between small-for-gestational-age (SGA) and appropriate-for-gestational-age (AGA) infants of various gestational ages (23–42 weeks) and those from term infants and adults. There was no significant difference between the absolute numbers or proportion of total T cells, helper or inducer T cells, or suppressor or cytotoxic T cells in preterm infants compared with term infants.¹⁴⁷ Series et al¹³³ studied the T-cell subsets in 104 preterm infants (36 healthy preterm infants and 68 preterm infants with perinatal distress) and 21 normal term infants in the first week of life. Significantly higher numbers of both CD2+ and CD4+ T cells that correlated with gestational age were reported. The absolute number of CD8+ T cells did not show differences

Table 1. CHARACTERISTICS OF NEONATAL T CELLS

Phenotype	85% express CD38 (<5% in adult)
CD4+ T cells	80%–90% express CD45RA+ or naïve phenotype (40%–60% in adult)
CD4+ T cells	<10% express CDw29
Circulating T-cell number	Higher than in adults at birth, peaks at 6–9 months of age, and attains adult values at 6–7 years of age CD4+ T-cell proportion and number are higher than in adults CD8+ T-cell proportion and number are lower than in adults CD4/CD8 ratio higher than in adults, reaches 2:1 ratio at 4 years
Proliferative responses	Comparable to those in adults with mitogens (e.g., PHA, Con-A, preterm < term) Stimulation with anti-CD2 and anti-CD3 MoAb less than in adult T cells Diminished MLR response; attain adult levels at 12 months of age Highly susceptible to tolerance induction by superantigens
T- and B-cell collaboration	
Contact-dependent	Diminished CD40 ligand expression, unregulated to adult levels after activation Decreased expression of CD54/ICAM-1, CD58/LFA-3
Cytokine production	Normal IL-2 but diminished IL-6, IL-4, and IFN- γ production
Specific cytotoxicity	30% of perforin expression compared to that in adults Significantly impair allogeneic CTL activity
LDCC	40% of adult T-cell activity
$\gamma\delta$ T cells	Reach adult repertoire level by 14–17 years of age Less cytolytic activity than adults

MoAb = monoclonal antibody; MLR = mixed lymphocyte culture reaction; CTL = cytotoxic T-cell lymphocytes; LDCC = lectin-dependent cellular cytotoxicity.

with gestational age. Preterm infants with perinatal distress had significantly lower numbers and proportions of CD2+ and CD4+ T cells. These changes were transient because the values taken at 3 weeks of age were comparable to those of healthy preterm infants. These markers remained lower in a group of preterm infants of less than 28 weeks gestational age in whom evidence of distress still persisted.¹³³ Ballow et al⁵ studied the chronologic changes of peripheral blood T-cell subpopulations in 36 very low birth weight (VLBW) infants for 6 months after birth; they showed, as expected, that there was significantly higher absolute numbers of CD3+ and CD4+ T cells compared with those in adult controls. There was also no correlation between the administration of steroids to the mothers before delivery to enhance fetal lung maturation and the percentages or absolute numbers of CD4 or CD8 or the CD4/CD8 ratio.⁵

Peripheral T-Cell Phenotypes

Several studies suggest that the peripheral T cells in the fetus and neonate represent an immature transitional T cell. Approximately 85% of cord blood T

cells express the CD38 surface marker compared with less than 5% of adult cells.¹⁵⁵ These findings suggest that cord blood T cells are either activated or at a late stage of intrathymic differentiation. Subsequent studies failed to demonstrate activation antigens (e.g. Ia or Ta1 antigen) on cord blood T cells. Thus cord blood T cells that express CD38 probably represent an immature population of T cells with different functional capacity than adult T cells.^{151, 154}

The expression of different isoforms of the T200 common leukocyte antigen (CD45) by human T cells delineates different functional subsets of T cells. T cells that express the higher molecular weight isotype, CD45RA, have a "naive" profile, whereas the T cells that express the smaller isoform of CD45 (e.g., CD45RO) have "memory" functions.^{32, 100} T cells in the thymus express CD45RA. This phenotype is also expressed on 80% to 90% of cord blood T cells, compared with 40% to 60% on adult cells.^{59, 63, 114} After mitogen stimulation, the CD45RA + T cells can change to the CD45RO + phenotype after 2 to 3 days, and by 6 days, most express CD45RO. These "primed" T cells are less dependent on costimulatory signals from accessory cells, which are necessary for naive T cells activation. Hayward et al have shown that the percentage of memory T cells (e.g., CD3 + CD45RO +) in healthy infants increases (Fig. 2) during the first few years of life to plateau after puberty and reaches adult levels after puberty.^{63, 114}

The CDw29 surface marker on T cells is augmented after stimulation and is found in high density on memory T cells.^{1, 124} Pirenne et al¹¹⁴ demonstrated a gradual increase in the expression of CDw29 on cord blood T cells after birth. At birth, CD4 + T cells express CDw29 on less than 10% of T cells and the CDw29 + CD4 + T cells increase during the first year of life, similar to the CD45RO isoform (see Fig. 2). Adult proportions of CD45RA/RO and CDw29 T-cell subpopulations are reached only during the second decade of life.¹¹⁴ Other markers that have been shown to change with age are CD28 and CD11b. Hoshino et al⁷⁰ investigated the distribution and age-related alterations in the expression of CD11b on human T cells and found a gradual increase in CD11b +

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Figure 2. Age-related changes in the expression of CD4 + T cells with the CD45RA + phenotype (naïve T cells) (*solid line*) and the CD45RA-CDw29 + phenotype (memory T cells) (*dashed line*). (Adapted from Pirenne H, Aujard Y, Eljaafari A, et al: Comparison of T-cell functional changes during childhood with the ontogeny of CDw29 + and CD45RA + expression on CD4 + T cells. *Pediatr Res* 32:81, 1992; with permission.)

T cells with age from 6% in newborns to adult levels of 25% to 27%. The expression of CD11b antigen occurs primarily on the antigen-specific memory T cells.⁷⁰ In contrast, CD28+ T cells decreased from 96% in newborns to 71% to 72% in adults.⁷⁰

Neonatal T cells express a "naive" phenotype of CD45RA+CDw29 that gradually converts to a memory phenotype, CD45RO+CDw29+ following exposure to their cognate antigens and activation after birth. Although a precursor-product relationship is suggested by previous observations, this pathway may not be unidirectional because some CD45RO+ T cells appear to change back to CD45RA+ in vivo.¹⁰⁰ The clear demarcation of these T-cell phenotypes and their function as naive or memory T cells are still under investigation.

T-Cell Function

Proliferative Responses to Mitogens, Antigens, and Allogeneic Cells

Phytohemagglutinin (PHA) and concanavalin-A (Con-A) are among the most widely used mitogens as nonspecific T-cell stimulators. The first PHA response by thymocytes is observed at 10 weeks' gestation and 3 to 4 to weeks later in the spleen and peripheral blood. The first response to Con-A is found in the thymus at 13 to 14 weeks gestation and in the fetal spleen at 14 to 18 weeks' gestation. At 14 weeks, the Con-A response was also detected in the liver and bone marrow.⁵⁹ As discussed by Toivanen et al¹⁴⁸ in his review, the PHA responses are stronger and develop earlier than the Con-A responses during T-cell ontogeny in humans. These findings suggest that there is a migration of mitogen-responsive cells from the fetal thymus into the spleen and other secondary lymphoid organs. Leino et al⁸⁷ studied the PHA and Con-A responses of cord blood cells from premature infants born at 25 to 36 weeks' gestation (birth weight <1000 g) using the whole blood culture method. The responses to PHA and Con-A at all concentrations were significantly less compared with that of infants with a birth weight greater than 2500 g.⁸⁷ The mitogenic responses in healthy, term neonates are comparable to those of adults.^{33, 114}

Proliferative responses to allogeneic cells can be evaluated by mixed lymphocyte reaction (MLR). The earliest MLR was identified by Stites¹⁴⁰ by the fetal liver cells at 7.5 weeks gestation, although this finding may represent a nonspecific response.¹⁴³ A MLR from the cells of the thymus, spleen, and peripheral blood can be identified at 11 weeks' gestation. After birth, the MLR responses of cord blood leukocytes^{33, 114, 122} appears to be strong, although somewhat less than that of adult peripheral blood until 12 months of age.

Neonatal lymphocyte proliferative responses to monoclonal antibodies (MoAb) of various T-cell surface determinants (e.g., CD3 and CD2) is dramatically less than that of adults. The response did not depend on the concentrations of MoAb or the time required for incubation. This diminished response is also characteristic of naive adult T cells. Responses increased with the increase in the proportion of peripheral blood memory T cells (e.g., CD45RO+). These findings suggest that the pattern of response to stimulation with CD2 and CD3 MoAb by neonatal lymphocytes is caused by the large proportion of naive T cells in the peripheral blood after birth.

The response of T cells to specific antigens can be assessed by either proliferation or cytokine (e.g., interleukin (IL)-2, IL-4, or interferon (IFN)- γ) production. These responses usually require previous exposure to the corres-

ponding antigen and are generally not detected at birth or from cord blood unless there was an intrauterine exposure. There have been reports, however, that neonatal T cells can respond to certain antigens (e.g., tetanus or diphtheria toxoids and influenza virus antigen).³³ Some findings could not be reproduced or indicated the transfer of maternal T cells across the placenta.¹⁰⁸ Clerici et al³³ clearly showed that cord blood T cells from 24 neonates born at 28 to 42 weeks' gestation had no proliferative response or IL-2 production to tetanus toxoid and influenza A virus until around 12 months of age.³³ Recently, Ferreira et al⁴⁷ reported a prospective study of 30 low birth weight (LBW) newborns, weighing 2000 to 2499 g (70% were SGA), and 56 control newborns weighing more than 2500 g, 1 to 5 days old who were immunized with intradermal Calmette-Guérin bacillus (BCG), the Moreau-Rio de Janeiro strain. Using lymphocyte proliferative responses to purified protein derivative (PPD) and measuring IL-2 activity with a CTLL-2 proliferative assay following 12 to 24 weeks after BCG administration, no significant differences in proliferative responses or IL-2 activity between the LBW infants and mothers were seen.⁴⁷

Superantigens are antigens that bind directly to MHC class II molecules, outside the TCR, and activate T cells that bear certain V β TCR. Takahashi et al¹⁴⁵ compared the immunologic reactivity of cord blood T cells and adult peripheral blood T cells with the toxic shock syndrome toxin-1 (TSST-1) superantigen. After 24 to 48 hours of incubation, cord blood T cells demonstrated slightly weaker TSST-1-induced IL-2 production than adult T cells. The percentage of V β 2+ T cells, which determine the potential reactivity to TSST-1, and the number of CD45RO+ T cells are increased significantly in both cord blood and adult peripheral blood T cells after stimulation. Interestingly, upon re-stimulation with TSST-1, cord blood T cells show no or very low IL-2 production compared with adult T cells that still react vigorously. These investigators concluded that the TSST-1-induced cord blood T cells were in an anergic state, whereas the adult T cells were sustained in a highly responsive state. This susceptibility to tolerance induction of cord blood T cells may be caused by their immunologic naivete.¹⁴⁵ It is not known how long this susceptibility to tolerance induction continues after birth.

Development of Cytokines

Cytokines are groups of small proteins that are secreted by a variety of cells including leukocytes, endothelial cells, fibroblasts, and other cell types. Cytokines are produced after activation of the secreting cells and exert their effects in the effector phases of both the innate and specific host immune responses. This section discusses the current knowledge of cytokines produced by neonatal mononuclear cells (T, B, and natural killer cells). Neonatal cytokine development is summarized in Table 2.

Using mitogens, lipopolysaccharides (LPS), or allogeneic cells, several investigators have demonstrated that neonatal IL-2 production of cord blood mononuclear cells (CBMC) is similar or higher than that in adult cells.^{42, 78, 91, 123} The high affinity IL-2 receptor is expressed normally on activated neonatal T cells and functions well in IL-2-induced proliferation, although neonates express less IL-2 γ .^{102, 160}

Production of the proinflammatory cytokines have been studied in the neonate. After lipopolysaccharide, PHA or Con-A stimulation of CBMC IL-6 production is normal^{90, 104, 125, 157}; however, neonatal monocytes produce only half the levels of IL-6 as adult monocytes.¹²⁸ CBMC produce normal amounts of IL-1 β after polyclonal activation but only one third to one half of the TNF- α levels of adults.^{27, 43, 125} Mitogen-activated neonatal T cells produce normal amounts of

Table 2. CYTOKINE DEVELOPMENT IN THE FETUS AND NEONATE

Cytokine	Cell Source	Target Cell	Comment*
Pro-inflammatory cytokines			
IL-1	mφ	Muscle, fat, endothelium, hypothalamus	Comparable to adults Increased with neonatal sepsis
IL-6	T cell, endothelial cell, mφ	Mature B cell, liver, thymocyte	Comparable to adults Increase serum levels after surgery and neonatal sepsis Cord monocytes: ~ 40%-50% of adult monocytes with LPS or RSV stimulus
TNF-α	mφ, T cell	Neutrophil, liver, endothelial cell	Comparable to decreased depending on the stimuli Increased serum levels with sepsis
IFN-α	mφ, other	NK cell, all	Cord monocytes: preterm < term and adults (LPS stim)
IFN-β	Fibroblast, other	NK cell, all	Normal in term infants; reduced in preterm infants Normal
Cytokines that regulate and mediate specific immunity			
IL-2	T cell	T, B, NK cell	Comparable to higher (T cells)
IL-4	CD4+ T cell, mast cell	B, T cell, endothelial cell	Very low to undetectable
IL-5	T cell	Eosinophil	Decreased
IL-10	mφ, T cell	mφ, B cell	Range between 15%-50% of adult depending on stimuli
IFN-γ	T, NK cell	All, endothelial cell, mφ	<10% of adult
TGF-β	T cell, mφ, other	T cell, other	30%-50% of adult
Lymphotoxin	T cell	Neutrophil, endothelial cell	Similar to higher compared with adults
Hematopoietic growth factors			
IL-3	T cell, mφ	Immature progenitor	Decrease to comparable to adult
GM-CSF	T cell, mφ, fibroblast, endothelial cell	Immature and committed progenitor, mφ	~50% of adult
G-CSF	mφ, endothelial cell, fibroblast	Committed progenitor	Cord monocyte: similar to adult
M-CSF	mφ, endothelial cell, fibroblast	Committed progenitor	Slight decrease or similar to adult
Chemokines			Preterm ~ term
IL-8 (CXCL8)	mφ, endothelial cell, fibroblast	Neutrophil	Similar to adult
MIP-1α (CCL3)	T cell, mφ	Monocyte, T cell, eosinophil	Normal (LPS stimuli) 14%-19% of adult

*Analysis tested in comparison with adult peripheral blood mononuclear cells and cord blood mononuclear cells (mixed population of T, B, and NK cells, and monocytes unless specified.

LPS stim = lipopolysaccharide stimulation of monocytes; mφ = monocytes/macrophages.

lymphotoxin (TNF- β).⁴³ In response to stress such as bacterial sepsis or surgery, newborn infants can increase their plasma IL-6 and TNF- α levels, regardless of their gestational age at birth; however, these levels are still less than those seen in adults with stress.^{37, 107, 146} This impaired TNF- α production under stressful conditions may contribute to a lower febrile response in neonatal sepsis. IL-10 is a modulatory interleukin that inhibits cytokine secretion by macrophages and T cells. Compared with adults, CBMC from neonates can secrete only 20% to 50% of IL-10 after LPS stimulation.^{27, 82} Lower IL-10 production may, in part, be secondary to the low TNF- α production of cord blood because TNF- α is known to enhance IL-10 secretion.^{27, 132} IL-15 is an important cytokine that regulates T-cell growth, T-cell cytotoxicity, and natural killer (NK) activity. From activated CBMC, IL-15 mRNA expression and protein production was 25% and 30%, respectively, at 12 hours of culture compared with adult peripheral blood mononuclear cells.¹¹⁶ The addition of exogenous IL-15 to CBMC cultures significantly enhanced cord blood NK and lymphokine-activated killers (LAK) activities and also significantly induced greater amounts of IFN- γ and TNF- α production. These investigators suggested that the reduced production of IL-15 may contribute to abnormalities in cytotoxic activity and diminished levels of the production of other cytokines such as IFN- γ and TNF- α .¹¹⁶

Hematopoietic colony stimulating factors (CSF) are growth factors for the proliferation and differentiation of myeloid progenitor cells, and they enhance mature neutrophil function.^{31, 134} Cairo et al^{17, 18} have reported reduced mRNA expression and protein production of several hematopoietic growth-inducing cytokines, including granulocyte-macrophage colony stimulating factor (GM-CSF), granulocyte CSF, macrophage CSF, and IL-3. Decreased mRNA expression was secondary to alterations in post-transcriptional events such as mRNA stability.^{85, 144} Others have reported that neonatal mononuclear cells produce similar or higher levels of IL-3 after lipopolysaccharide stimulation but significantly lower levels of IL-3 after PHA, anti-CD2, or anti-CD3 antibody stimulation.^{115, 125} GM-CSF production by full-term neonates is also less than in adults.²⁰ Several cytokines are negative regulators of hematopoiesis, including transforming growth factor β 1 (TGF- β 1) and macrophage inflammatory protein-1 α (MIP-1 α). Cairo et al proposed that altered levels of these cytokines might influence hematopoiesis. Using activated CBMC, TGF- β production was only 30% to 50% of adult levels, and the synthesis of the chemokine, MIP-1 α , was only 14% to 19% compared with adult cells.²⁵ Based on the lower levels of mRNA expression, but a normal nuclear run-on assay and quantitative densitometry, it was suggested that this decrease in TGF- β and MIP-1 α was secondary to altered post-transcriptional regulation.²⁵

Lewis⁸⁹ and others^{62, 101, 115, 153} have demonstrated that unfractionated or purified neonatal T cells after polyclonal activation produce negligible to undetectable amounts of IL-4 and less than 10% of adult levels of IFN- γ . The decrease in mRNA expression was primarily caused by reduced transcription of the IFN- γ and IL-4 genes.⁸⁹ IL-4 and IFN- γ at both the protein and mRNA level are largely produced by the CD4+ CD45RO+ T-cell subpopulation.^{62, 89} Both activated neonatal CD4+ and adult CD45RA+ T cells produce low to undetectable IL-4 and markedly low IFN- γ mRNA.⁸⁹ As the number of CD45RO+ T cells increases, IFN- γ production also increases. It remains to be determined whether this correlation also applies to IL-4 production.⁸⁹ These findings suggest that the decrease in the production of these cytokines is caused by the lack of memory or primed T cells. IL-4 production reaches adult levels after 15 years of age.¹⁴⁶ IFN- γ reaches adult levels at the age of 3 or later.¹⁰¹ Piccinni¹¹³ investigated the production of IL-4 and IL-5 from newborns with atopic parents. Interestingly, in

neonates with atopic parents or only an atopic mother, T-cell clones produced significantly higher amounts of IL-4 and IFN- γ , compared with T-cell clones from neonates with normal parents or only an atopic father. Some of the newborns from atopic parents or an atopic mother developed atopic manifestation and elevated serum levels of IgE before 3 years of age. These findings suggest that maternal genetic factors are more influential than paternal genetic factors in predicting the risk for developing atopy. The genetic background of the neonate may determine a preferential shift to a Th2 phenotype and probably dictates the response to common environmental allergens.¹¹³ These observations need further study.

The neonate produces normal levels of IL-2 and lymphotoxin at birth; however, the production of a number of proinflammatory cytokines, hematopoietic colony stimulating growth factors, and immune regulating cytokines (IL-3, IL-4, IL-5, IL-6, IL-10, TNF- α , IFN- γ , GM-CSF, MIP-1 α , and TGF- β) from CBMC are decreased (see Table 2). Most of these deficiencies are caused by altered post-transcriptional mRNA regulation. The increased prevalence of naive CD45RA + T cells compared with primed or memory CD45RO + T cells in the neonate reflects an antigenically naive status and may account for these deficiencies in cytokine production rather than an intrinsic deficiency in the capacity of newborn CBMC to produce cytokines.⁸⁸

T- and B-Cell Collaboration

For B lymphocytes to proliferate, differentiate, switch antibody isotypes, and survive in germinal centers, they essentially need to interact with their activated, cognate antigen-specific T lymphocyte. The first signal delivered to the T cell is the ligation between the peptide fragments of the antigen and the TCR coupled with MHC class II molecules. T cells also provide additional help by contact-dependent interaction and the release of cytokines (IL-2, IL-4, IL-5, IL-6, IL-10, IL-13, and IFN- γ) to promote B-cell proliferation and differentiation. Several pairs of receptor-ligand molecules are crucial in T- and B-cell collaboration. For example, the CD40-CD40 ligand (CD40L) interaction is important for B cell isotype switching.^{30, 34, 38} Other ligand pairs that promote interaction and enhance T- and B-cell activation include B7/CD28, CD11a (LFA-1)/CD54 (ICAM-1), and CD58 (LFA-3)/CD2.³⁰

CD40L is a 39 kD type II membrane glycoprotein of the TNF-receptor superfamily and is expressed on activated T cells (mostly on the CD4 + subset, although some CD8 + T cells also express CD40L), mast cells, basophils, NK cells, monocytes, and on some B cells.⁹⁶ Brugnani et al,¹⁵ using anti-CD40 L MoAb, and later confirmed by Nonoyama¹⁰⁵ using a CD40L-Ig binding assay, consistently demonstrated that activated neonatal T cells had significantly less CD40L expression. When looking at the relationship of CD40L expression on T cells, more than 80% of adult CD45RA + T cells express CD40L compared with less than 30% of neonatal CD45RA + T cells.¹⁰⁵ These results suggest that neonatal CD45RA + T cells are different than adult CD45RA + T cells. After priming with PHA and IL-2, which has been shown to convert "naive" T cells to CD45RO + "memory" T cells, neonatal T-cell CD40L expression is upregulated to levels comparable to those of adult T cells.^{15, 105}

The capacity of neonatal B cells to differentiate into plasma cells and undergo isotype switching can be variable. Cord blood B cells in the presence of adult CD4 + T cells stimulated with immobilized anti-CD3 produces only IgM.^{2, 39, 95, 101, 138, 139} The differentiation of B cells into plasma cells and the process of isotype switching require CD4 + T-cell help that is provided by two important

signals. The first signal is mediated by contact between the helper T cell and the B cell through the CD40 ligand on activated T cells as well as other secondary signal interactions (e.g., CD28 and B7). The second important aspect of T-cell help is provided by cytokines that are secreted upon T-cell activation. Splawski and Lipsky¹³⁸ reported that neonatal B cells incubated with *Staphylococcus aureus* and supernatant from immobilized activated adult T cells enhanced only IgM production. When IL-2 is added to neonatal B cells, switching to IgG and IgA isotypes occurs, although modestly.¹³⁸ When either IL-4 or IL-6 is added to immobilized anti-CD3 activated neonatal T cells with neonatal B cells in the presence of IL-2, however, the production of all Ig isotypes, including the various IgG subclasses, IgA and IgE are enhanced.¹³⁹ In this in vitro culture system, IL-2 appears to be critical for Ig secretion and isotype switching of neonatal B cells. Both IL-4 and IL-6 enhance Ig production in an IL-2-dependent manner.¹³⁹ Kawano et al⁷⁷ found that IFN- γ induced a limited but substantial enhancement of IgG2 secretion. Interestingly, after stimulation by anti-CD3 with or without cytokines, neonatal T cells maintained a CD45RA+ phenotype, suggesting that the T-cell help in this system was provided by naive neonatal T cells, and differentiation into CD45RO+ CD4+ T cells does not occur.¹³⁹ Neonatal T cells also provide less contact-dependent interaction for other ligands (e.g., CD54 or ICAM-1 and CD58 or LFA-3).⁵⁵

The deficiencies in Ig-secreting B cells and isotype switching are caused by, in part, the naive state of neonatal B cells but can be induced to isotype switch given the proper T-cell-derived cytokines. The immaturity in T-cell help stems from a lack of memory and a predominance of naive neonatal T cells. In addition, the poor Ig production of neonatal B cells appears to be caused by diminished T- and B-cell interaction because of lower levels of CD40L on activated neonatal T cells that underscores the importance of CD40L for inducing isotype switching in B cells.¹⁰⁵ It remains to be determined whether these in vitro experimental findings reflect the in vivo biology of T- and B-cell collaboration.

ONTOGENY OF CYTOTOXIC T LYMPHOCYTES.

The majority of cytotoxic T lymphocytes (CTL) express CD8 on their surface, although CD4 can be detected on a small number of CTLs.⁹² CTLs recognize their cognate antigens in the context of MHC class I molecules. CTLs are important in host defense against a variety of intracellular infections, bacteria and viruses, in allograft rejection, and in tumor cell surveillance. CTLs exert these killing functions by two well-established mechanisms. The first mechanism involves the release of mediators, perforin (cytolysin), which is the pore-forming protein, and a serine protease enzyme called granzyme B. The perforin by itself is usually enough to cause osmolysis of the target cell. More importantly, it provides a pathway for the granzyme B to get into the target cell, activating a cascade of events that leads to the apoptotic death of target cells similar to apoptosis induced by the Fas/Fas ligand pathway.⁷⁴ Activated CTLs can also kill cells through the Fas/Fas ligand pathway that leads to target cell apoptosis.⁷⁴

Cell-mediated lympholysis (CML), using allogeneic cells as the inducer, can be detected by 18 weeks of gestation in human peripheral blood lymphocytes obtained from fetuses through a fetoscope.¹⁴⁸ Postnatal cord blood allogeneic CTL activity is less than 20% of the CTL activity of adult cells.¹²² Neonatal CD8 T cells have less perforin expression, approximately 30% of adult levels (see Table 1).⁸⁸ CD8+CD28+ T cells are considered to be the effector cells responsible for CTL activity in adults. CD28, expressed on activated T cells, is a co-stimulatory

molecule that enhances T-cell function when bound to its ligand, B7-1 or B7-2. Cord blood CD8⁺ T cells express significantly less CD28⁺ than adult CD8⁺ T cells after stimulation.¹²² This impaired allogeneic CTL function in cord blood may explain the lower incidence of graft-versus-host disease after cord blood transplantation compared with bone marrow, even with partially matched cord blood mononuclear cells.

Lectin/mitogen-dependent cytotoxicity (LDCC) can be detected as early as 14 weeks of gestation in the fetal spleen, bone marrow, and peripheral blood, and 2 weeks later in fetal thymus.¹⁴⁸ Cord blood LDCC activity is only 40% that of adult activity (see Table 1). Interestingly, Lubens et al⁹⁴ found that LDCC activity in the peripheral blood of 1 to 4 day old neonates was only 33% that of adult LDCC activity. This difference in LDCC between cord blood and neonates may be secondary to the release of inhibitory mediators yet to be identified during the mechanical trauma of delivery.⁹⁴ LDCC activity increases after 6 months of life.

It has been postulated that deficiency in CTL function during the neonatal period is caused by several contributing factors. High levels of certain mediators in cord blood, such as α -fetoprotein and certain prostaglandins, have been shown to inhibit CTL activity *in vitro*.⁹⁴ As mentioned previously, the perforin deficiency may also lower the CTL function of neonatal T cells.

NK CELL ONTOGENY

NK cells are derived from the bone marrow. These cells are called large granular lymphocytes because of their larger size and numerous cytoplasmic granules. These granules are known to contain perforin, granzyme, and proteoglycans. NK cells are considered to be a distinct subset of lymphocytes (e.g., non-T and B cells) because they express no surface CD4, CD8, Ig, or TCR and do not undergo TCR or Ig gene rearrangement during their differentiation. NK cells can be identified by their surface markers, CD56/neural cell adhesion molecule, and CD16/Fc γ RIIIA, a low affinity IgG receptor. They also express other surface molecules, including CD2, LFA-1, cytokine receptors (e.g., IL-2R $\beta\gamma$, IL-12R, IFN- γ R), and the IL-15R α subunit. These cytokines enhance NK cytolytic activity. IL-2, IL-7, and stem cell factor (SCF) may be involved in NK development.¹³⁷ NK cells recognize their target cells by the absence or decreased expression of MHC class I molecules on the cell surface, which is known to be one of the several mechanisms that certain viruses use to manipulate host immune response. Thus NK cells are important in nonspecific host defenses against viral infections and some tumor cells. Their MHC-unrestricted killing is mediated by perforin/granzyme apoptotic pathways similar to that of CTLs. Another killing mechanism of NK cells is antibody-dependent cell-mediated cytotoxicity or ADCC in which the Fc γ RIIIA receptor is triggered by IgG1 or IgG3 that is bound on the target cell surface. NK cells can differentiate into more efficient cytolytic cells LAK, in the presence of cytokines such as IL-2 and IL-12.

Several lines of evidence suggest that NK cells may originate from common T/NK progenitors. First, some patients with severe combined immunodeficiency (SCID) have normal or a high number of B cells and myeloid cells but lack T and NK cells.¹³⁷ Second, the majority of fetal NK cells (50%–80%) express cytoplasmic CD3 γ , CD3 ϵ , CD3 δ , and CD3 ζ proteins but only CD3 ζ is expressed in adult NK cells.¹³⁷

The phenotype of fetal NK cells is different from that of adults (Table 3). Of fetal NK cells, 50% to 80% express CD3 γ , ϵ , δ , and ζ proteins; a small number

Table 3. CHARACTERISTIC OF NEONATAL NK CELLS

Phenotype	Function
Only ~50% express CD56	Spontaneous cytotoxicity only 20%–60% of adult activity
Lack CD57 expression	Diminished ADCC: ~50%–80% of adult activity at term
Reduced CD16 expression than in adults	Comparable or higher LAK induction by IL-2

ADCC = antibody-dependent cellular cytotoxicity; LAK = lymphokine-activated killer cells.

of cord blood NK cells also express CD3 ϵ , δ , and ζ proteins, but only CD3 ζ is identified in mature adult NK cells.¹¹² Only 30% to 50% of the fetal NK cells express CD16 on their cell surface compared with more than 90% of neonatal and adult NK cells.¹¹² CD57 is not found on both fetal and neonatal NK cells, although CD57 can be detected on 50% of adult NK cells.¹¹² Similarly, CD56 is not detected on fetal cells but is detected on only 50% of neonatal NK cells compared with that in adults.^{50, 112} NK cells can be detected as early as 6 weeks of gestation in the fetal liver and at 15 weeks of gestation in the fetal spleen.¹¹² The number of NK cells increases progressively until birth. Of cord blood lymphocytes, 10% to 15% are NK cells, which is comparable to the frequency of NK cells found in adult peripheral blood.^{88, 112}

Cytolytic activity against NK-sensitive tumor cell targets (K562 or Jurket) from freshly isolated fetal NK cells is significantly less than that of adults and increases with gestational age.^{99, 112} Neonatal NK cells have decreased cytolytic activity compared with adult NK cells and appear to parallel the number of CD56+ NK cells in the peripheral blood of neonates.^{28, 88, 94, 112, 148} The cytotoxicity of these immature NK cells increases as their expression of CD56 increases.^{88, 137} Fetal and neonatal NK cell function can be augmented when co-cultured with IL-2, IL-12, or IFN- α , IFN- β or IFN- γ in vitro.^{28, 88, 112, 148} ADCC activity of fetal NK cells is less than that of neonatal and adult NK cells.^{88, 112} The ADCC activity increases with gestational age and reaches approximately 50% to 80% of adult values depending on the purification and culture techniques.^{88, 112} This decrease in ADCC is likely to be secondary to the lower expression of CD16 (see Table 3).

Fetal NK cells may actually develop from common, bipotential T-cell/NK progenitors and branch out to become committed to NK cells, probably at the triple-negative thymocyte stage. All fetal and some neonatal NK cells lack CD57 and express lower levels of CD56 and CD16 molecules on their cell surface. The cytolytic activity increases as the CD56 and CD16 expression increases.

DEVELOPMENT OF B CELLS AND SERUM IMMUNOGLOBULINS IN THE NEONATE

Ontogeny of Fetal and Neonatal B Cells

In humoral immune responses, B cells and plasma cells are the main effector cells that mediate their functions by producing antibodies of different Ig isotypes. The surface Igs (sIg) are the antigen receptors for B cells. Similar to T-cell development, B cells need to pass through different stages of maturation and differentiation involving Ig gene rearrangements and changing cell membrane molecules of surface ligands and receptors. B-cell progenitors, pro-B cells, are

derived from pluripotential hematopoietic cells in the bone marrow. The first recognizable B-cell progenitor is the large pre-B cell that contains cytoplasmic μ heavy chain that may be associated with invariant surrogate light chains (e.g., VpreB and $\lambda 5$) that are homologous to the κ and λ light chains.⁵⁶ Low-level cell surface expression of μ heavy chain and the surrogate light chains is thought to play a role in the maturation of these pre-B cells. Ig α and Ig β function as signal transduction subunits and are required for the expression of surface Ig.^{56, 120} Once the light chain genes, either the κ or λ chains, are rearranged successfully, they assemble with the μ chains to form cell surface IgM (sIgM), where they can function as specific receptors for antigen. These sIgM+ B cells, called "immature B cells," leave the bone marrow to circulate in the periphery. B-cell development requires fetal liver, and later, the bone marrow microenvironment. Stromal cells on contact with developing B cells through adhesion molecules, VCAM-1, provide growth factors such as stem cell factor, IL-7, and other cytokines yet to be identified.⁷ Somatic hypermutation, a process that does not occur in T cells, is another important step to enhance the affinity of the B-cell receptor upon subsequent antigen exposure. Immature B cells go through a selection process similar to T cells that results in apoptosis (clonal deletion) or tolerance to self-antigens. Mature B cells co-express μ and δ heavy chains together with the original κ or λ light chain when they populate the peripheral lymphoid tissues.

In the human fetal liver, pre-B cells can be identified as early as 7 to 8 weeks of gestation and 5 to 6 weeks later in the fetal bone marrow.⁵³ By 11 to 16 weeks of gestation, large and small pre-B cells have outnumbered their progeny in the fetal liver.¹⁵² By 30 weeks of gestation, there is no detectable pre-B cell in the fetal liver, and the bone marrow becomes the exclusive site for pre-B-cell maturation. sIgM+ B cells are found in the fetal liver by 9 weeks' gestation and in the bone marrow, peripheral blood, and spleen by 12 weeks.⁵³ sIgM+ B cells expressing surface IgM are present by 10 weeks' gestation.⁵³ B cells with sIgA, sIgG, and sIgD isotypes appear between 10 and 12 weeks' gestation. B-cell traffic to the lymphoid tissues increases, and by 22 weeks' gestation the proportion of B cells in the spleen, peripheral blood, and bone marrow is similar to that in adults.⁵³ Unlike adult B cells that express only one Ig isotype (except for IgM/IgD+ B cells), many of these neonatal B cells express all three isotypes together. Plasma cells are not generally found until 20 weeks' gestation. IgM/IgD+ B cells populate the lymph nodes by 16 to 17 weeks' gestation and the spleen by 16 to 21 weeks. In fetal lymph nodes, primary nodules develop around the follicular dendritic cells by 17 weeks' gestation.¹²

After birth (Table 4), the proportion of neonatal B cells is similar to that of

Table 4. CHARACTERISTIC OF NEONATAL B CELLS

Phenotype	~90% of peripheral circulating B cells are CD5+ (25%–35% in adults), decreases to adult proportions in late adolescence
Circulating B-cell number	Similar proportion but higher absolute numbers than in adults, peaks at 3–4 months of age, and decreases to adult levels by 6–7 years of age
Function	Mostly IgM secreting; isotype switching impaired, caused by, in part, decreased neonatal T-cell help through CD40/CD40L interactions and diminished T-cell derived cytokines

adults, but the absolute number of B cells is significantly higher, peaks at about 3 to 4 months of age, and decreases thereafter to attain adult levels by 6 to 7 years of age.^{95, 109, 149} Preterm infants have comparable B-cell numbers to full-term infants, but SGA infants have significantly less B cells than AGA infants.¹⁴⁷ Uniquely, the majority of cord blood B cells express activation markers (CD25, CD23, transferrin receptor, and others) that are not found on adult B cells.⁴⁰ The significance in the expression of these activation markers on neonatal B cells is not known.

The Immunoglobulin Repertoire in the Fetus and Neonate

The genes for the Ig heavy chain are located on chromosomes 14; the genes for the κ and λ light chains are located on chromosomes 2 and 22, respectively. For the Ig heavy chain, gene segments from the variable (V), diversity (D) and joining (J) regions are spliced together to form the antigen-binding site (see Fig. 1). Each region is encoded by its own set of germ line gene loci (e.g., V_H gene region of approximately 100 or 50 functional genes), V_D region of approximately 30 genes, and the V_J region of 6 genes.⁹⁷ Receptor diversity in the antigen binding site originates from the DNA recombinatorial process of various V, D, or J gene segments coming together to form a V(D)J unit¹²⁵ (see Fig. 1). Additional receptor diversity is generated by imprecise gene segment joins, additional nucleotides added to the splice junction of the VDJ joins by the enzyme TdT, and somatic mutations (for B cells only, not T cells). Thereafter, these VDJ or VJ (light chains) units join to their respective constant region gene segments (V_H) that for the immunoglobulin molecule identifies the isotype and related functions. As recently reviewed by Schroeder and Perlmutter,¹³⁰ the immunoglobulin repertoire in the fetus and neonate is restricted compared with that of adults. During early and mid-gestation, the fetal use of certain V heavy gene segments are preferentially expressed.^{111, 129} The most J_H proximal V_H gene segments are utilized more commonly in preferential V_H rearrangements early in fetal life.¹³¹ The CDR3 region of the rearranged VDJ gene segment is shorter than that in adults and leads to limited junctional diversity. These findings are secondary to lower levels of TdT enzyme, as discussed previously,¹¹⁷ or other mechanisms under developmental control. The B-cell repertoire progressively increases with gestational age with changing utilization of certain V_H gene families. Cord blood B cells have a higher usage of V_H genes of the V_{H1} and V_{H5} family with decreased V_{H3} use compared with adult B cells.²⁹ The antibody response of the infant is generally of low affinity and restricted to the IgM isotype. The somatic mutation of the heavy and light Ig variable region genes and the selection of higher affinity antibody-producing B cells is limited at birth but increases by 10 days of age¹²¹ and reaches adult levels by age 4. The onset of somatic mutation can precede isotype switching within the germinal centers.^{93, 121}

Schroeder et al¹³¹ have suggested that limitations in the size of the CDR3 domain will result in a "flat" antibody-binding site. This architecture of the antigen-binding site allows greater polyspecificity of antigen binding, resulting in lower antibody affinity. The clinical significance of this restricted Ig repertoire and the prevalence of lower affinity antibodies in the neonate with regard to the susceptibility of the neonate to infection and the ability to produce specific antibody needs further study.

Development of CD5 Expressing B Cells

CD5 is typically expressed on T cells. B cells with surface expression of CD5 represent a functionally distinct subset of B cells. They are equivalent to B1 or Ly-1 B cells in the mouse. Most of the CD5⁺ B cells express sIgM^{bright}sIgD^{dull},⁷⁵ and produce mostly low affinity IgM polyspecific, autoreactive antibodies such as rheumatoid factor (RF), anti-ssDNA, anti-thyroglobulin antibodies, compared with the high affinity antibodies of conventional B cells.^{21, 22, 75} CD5⁺ B cells use a restricted set of V heavy genes.¹¹⁹ The development of CD5⁺ B cells may precede that of conventional B cells in ontogeny because they comprise the majority of B cells during fetal life.^{3, 8} The CD5⁺ B cells lineage is still unknown, although several lines of evidence suggest that they may represent a separate lineage from that of CD5⁻ B cells (for critical reviews see references 22, 75). CD5⁺ B cells have a distinctive anatomic localization in the peritoneal cavity. The exact function of this B-cell subset is currently unclear. It has been postulated that CD5⁺ B cells may play a role in innate immunity because they have a unique localization, broad polyspecific specificities, and a restricted Ig repertoire.

CD5 expression can be found on about 1% to 7% of adult peripheral blood mononuclear cells and on 25% to 35% of adult total B cells.^{3, 8} CD5⁺ B cells comprise the majority of B cells in fetal and neonatal blood (see Table 4). CD5⁺ B cells are found as early as 15 weeks of gestation in the human fetal spleen and peritoneal and pleural cavities.^{8, 12} They comprise more than 90% of the total splenic B cells at gestational ages of 17 to 23 weeks⁸ but are infrequent in the fetal liver and adult spleen.³ In fetal lymph nodes between 17 and 18 weeks of gestation, CD5⁺ IgM⁺ IgD⁺ B cells form the first primary nodules.¹² Cord blood CD5⁺ B cells represent approximately 90% of the total B cells, decreases to 75% to 80% during infancy, and gradually declines from early childhood to reach adult levels of about 25% to 35% of total B cells or 1% to 7% of the total number of mononuclear cells by late adolescence.^{3, 8}

Serum Immunoglobulin Levels in the Fetus and Neonate

Despite the nearly normal number of sIg-bearing B cells in the blood and spleen by 15 and 18 weeks' gestation, serum levels of Ig are very low until approximately 18 to 20 weeks' gestation. Most of the newborn's serum immunoglobulins are derived from the transfer of maternal IgG across the placenta during the third trimester of pregnancy.⁸¹ Active transport of IgG across the placenta is mediated by the Fc receptor on placental cells. In the full-term neonate, serum IgG levels are equal or even higher than maternal serum IgG levels.⁸¹ Through normal catabolism of the maternal-derived IgG, the serum IgG level in a full-term infant falls to 300 to 500 mg/dL between 3 and 5 months of age, resulting in a physiologic hypogammaglobulinemia. Thereafter, as the infant makes his or her own IgG, serum levels increase to approximately 60% of adult IgG levels of 1200 mg/dL. There may be a preferential transport of IgG1 and IgG3 across the placenta because of a higher affinity of the Fc receptors on trophoblasts for these IgG subclasses.^{41, 98} There is also a differential progression in the synthesis of the IgG subclasses after birth. IgG1 and IgG3 reach adult levels by age 8, whereas IgG2 and IgG4 rise more slowly and do not reach adult levels until age 10 to 12.¹⁰⁶ Because the premature infant derives most of the IgG from the mother in the third trimester of pregnancy, the serum IgG levels are less than maternal levels and are directly related to gestational age.^{46, 66, 71, 159} Hobbs and Davis⁶⁶ found that nearly all infants born before 32 weeks' gestation

had serum IgG levels less than 400 mg/dL at birth compared with term infants who had serum IgG levels of approximately 1000 mg/dL. Because levels tend to fall for several weeks after birth, a high proportion of preterm infants develop significant hypogammaglobulinemia during the first 6 months of life. In a study by Ballow et al,⁶ preterm infants born prior to 29 weeks' gestation had profoundly low plasma IgG levels (geometric mean of 60 mg/dL) at 3 months of age. Even at 6 months of age, 7 of 11 infants born prior to 29 weeks' gestation and 13 of 21 preterm infants born between 29 and 32 weeks' gestation had plasma IgG levels less than 200 mg/dL. Plasma levels of IgG slowly increased to 280 mg/dL at 10 months of age.

Serum IgA can be first detected in fetal serum at 30 weeks' gestation, depending on the assay sensitivity. Because essentially no IgA crosses the placenta during pregnancy, serum IgA levels are very low, even in term infants. By 1 year of age, serum IgA levels reach only 20% of adult values. The same occurs for IgM and IgE, which do not cross the placenta; however, the fetus is capable of producing appreciable amounts of IgM as evidenced by the serum IgM levels following intrauterine infection.¹¹ IgM synthesis accelerates more rapidly than the other Ig isotypes, and reaches 80% of adult values by age 1. Cord blood levels of Ig in infants with intrauterine growth retardation are often lower compared with normal gestational age term infants.¹⁵⁸

Antibody Responses to Specific Antigens and Vaccines

Neonates also have a reduced capacity to produce specific antibodies in the newborn period. Smith immunized newborns with a killed *Salmonella* vaccine.¹³⁶ Both premature and term infants produced antibody to the flagellar H antigen, but not to the somatic O antigen. No infants switched from IgM to IgG anti-flagellar antibody for the first 20 to 30 days following immunization; normally adults convert by day 7. Preterm infants (<2300 g) immunized to diphtheria toxoid at 1 to 10 days of age had poorer responses than similarly immunized term infants; even term infants responded more slowly than adults.¹⁵⁰ Several studies suggested that the interval from birth is a more important determinant of antibody response than the gestational age. For example, Dancis et al³⁶ demonstrated that preterm infants immunized at their estimated normal birth age (at approximately 1 to 2 month's chronologic age) responded well to diphtheria toxoid. Both preterm and term infants had a poor antibody response when immunized at birth. Several studies have showed, however, that the immunization of preterm infants with hepatitis B vaccine led to lower seropositive rates and lower antibody titers than term infants. Lau⁸⁴ reported that preterm infants who received their first hepatitis B immunization when they weighed about 1000 g had a seropositive rate of 79% (geometric mean titer 61 mIU/mL) compared with term infants (100% seropositive rate; 679 mIU/mL). In a similar study in Thailand the seropositive rate was only 88% at 9 months of age.²⁶

Cates et al²⁴ measured the Ig response to vaccine challenge in VLBW infants. These premature infants were capable of producing specific IgG antibody in response to immunization with tetanus and diphtheria toxoids. By 8 to 11 months' chronological age, the preterm infants had developed levels of functional opsonic antibody activity for an IgG-dependent strain of coagulase negative staphylococcus and an IgM dependent strain of *Escherichia coli*, which were comparable to those of 9-month-old term infants. Interestingly, IgG antibody concentrations at birth in preterm infants to tetanus and diphtheria were compa-

rable to those of their mothers even though the total IgG levels were significantly lower than those of their mothers. Because most of the tetanus IgG antibody is IgG1, there is a preferential transport of the IgG1 subclass across the placenta to account for these findings. Although the preterm infants' tetanus and diphtheria IgG levels remained lower than those of the term infants after three doses of the vaccine, all preterm infants had protective levels of specific antibodies to tetanus and diphtheria toxoid. Using a functional antibody assay for opsonic activity, IgG coagulase negative staphylococcal activity fell from birth to 3 months of age, but by 9 months was comparable to term infants. Krediet et al⁸³ studied preterm infants who had coagulase-negative staphylococcal septicemia. IgG antibodies and opsonic activity were low at birth in infected and noninfected neonates. During the first 2 weeks of postnatal life, antibody levels decreased significantly in septicemic infants, whereas opsonic activity remained unchanged. IgM opsonic activity for *E. coli* is very low at birth in both term and preterm infants and rises with chronologic age with the rise in total plasma IgM to reach similar levels at 9 months of age. These studies show that preterm infants can produce specific and functional antibodies at levels similar, but lower than term infants by 9 months of age despite the profound hypogammaglobulinemia during the first 6 months of life in VLBW infants.

The response to polysaccharide antigens primarily falls within the IgG2 subclass, an IgG subclass that is one of the last to reach adult levels in children. Commensurate with this slow rise in IgG2 is the poor antibody response to bacterial polysaccharide antigens (e.g., *Haemophilus influenzae*, streptococcal pneumonia).⁵⁸ Responses to the unconjugated vaccine for these bacterial organisms are often delayed until after the age of 2. This physiologic delayed maturation in the IgG2 subclass and poor antibody responses to polysaccharide antigens may result in recurrent or persistent infections of the upper airways, such as sinusitis.⁵¹

SUMMARY

The susceptibility of neonates to infection clearly results from their immune deficiency in both the innate and specific immune host defense systems. The impaired neonatal T-cell functions are caused by the predominance of the CD45RA naive phenotype. The decreased CD40 L expression on CD4+ T-helper cells leads to diminished T- and B-cell collaboration that results in lower Ig production and poor isotype switching. The CBMC of neonates also produces less proinflammatory cytokines, hematopoietic colony stimulating growth factors, and immune regulating cytokines (IL-3, IL-4, IL-5, IL-6, IL-10, TNF- α , IFN- γ , GM-CSF, MIP-1 α , and TGF- β) compared with the peripheral blood mononuclear cells of adults. These deficiencies impact on other immune effector cells and leads to further compromises in other immune functions. Recent studies suggest that neonatal T cells have normal capacities but altered thresholds of responsiveness, in part, related to the naive phenotype of their CD4+ T cells. Cytotoxicity mediated by neonatal NK cells is diminished compared with that of adult cells. Neonatal NK cells lack CD57 and express lower levels of CD56 and CD16 (Fc γ RIIIA) determinants on their cell surface. The cytolytic activity increases as the CD56 and CD16 expression increases; however, in the presence of IL-2, neonatal NK cells transform effectively to LAK cells.

slg+ B cells can be detected early in gestation, and by 22 weeks' gestation normal numbers of B cells can be found in the peripheral lymphoid tissues. The immunoglobulin repertoire in the fetus and neonate is restricted compared with that of adults. The B-cell repertoire progressively increases with gestational age

with changing utilization of certain V_H gene families. The antibody response of the infant is generally of low affinity and restricted to the IgM isotype. CD5+ B cells comprise the majority of B cells in fetal and neonatal blood and produce mostly low affinity IgM polyspecific, autoreactive antibodies. However, intra-uterine infections can result in the production of Ig antibodies of all three major isotypes. Serum levels of Ig are very low until approximately 18 to 20 weeks' gestation. Most of the newborn's serum immunoglobulins are derived from the transfer of maternal IgG across the placenta during the third trimester of pregnancy. Between 3 and 5 months of age, serum Ig levels reach their nadir, resulting in a physiologic hypogammaglobulinemia. Neonates also have a reduced capacity to produce specific antibodies in the newborn period. By one year of age, most term infants can produce normal levels of specific antibodies to glycoprotein antigens; however, antibody responses to polysaccharide antigens are delayed until after age 2.

References

1. Akbar AN, Terry L, Timms A, et al: Loss of CD45R and gain of UCHLI reactivity is a feature of primed T cells. *J Immunology* 140:2171-2178, 1988
2. Andersson U: Development of B lymphocyte function in childhood. *Acta Paediatr Scand* 74:568-573, 1985
3. Antin J, Emerson S, Martin P, et al: LEU-1+ (CD5+) B cells. A major lymphoid subpopulation in human fetal spleen: Phenotypic and functional studies. *J Immunol* 136:505, 1986
4. Asma GEM, van den Bergh RL, Vossen JM: Use of monoclonal antibodies in a study of the development of T lymphocytes in the human fetus. *Clin Exp Immunol* 53:429-436, 1983
5. Ballow M, Cates KL, Rowe J, et al: Development of the immune system in very low birth weight (less than 1500 g) premature infants: Concentrations of plasma immunoglobulins and patterns of infections. *Pediatr Res* 20:899-904, 1986
6. Ballow M, Cates K, Rowe J, et al: Peripheral blood T-cell subpopulations in the very low birth weight (less than 1500 g) infant. *Am J Hematol* 24:85-92, 1987
7. Banchereau J, Rousset F: Human B lymphocytes: Phenotype, proliferation, and differentiation. *Adv Immunol* 52:125-262, 1992
8. Bhat N, Kantor A, Bieber M, et al: The ontogeny and functional characteristics of human B-1 (CD5+B) cells. *Int Immunol* 4:243-252, 1991
9. Bhutta ZA: Neonatal infections. *Curr Opin Pediatr* 9:133-140, 1997
10. Blackman M, Kappler J, Marrack P: The role of the T cell receptor in positive and negative selection of developing T cells. *Science* 248:1335-1341, 1990
11. Blankenship WJ, Cassady G, Schaefer J: Serum gamma-M globulin responses in acute neonatal infections and their diagnostic significance. *J Pediatr* 75:1271, 1969
12. Bofill M, Janossy G, Janossa M, et al: Human B-cell development, II-Subpopulations in the human fetus. *J Immunol* 134:1531, 1985
13. Bonati A, Zanelli P, Ferrari S, et al: T-cell receptor B-chain gene rearrangement and expression during human thymic ontogenesis. *Blood* 79:1472-1483, 1992
14. Borst J, Broom TM, Bos JD, et al: Tissue distribution and repertoire selection of human $\gamma\delta$ T cells: Comparison with the murine system. *Curr Top Microbiol Immunol* 173:41-46, 1991
15. Brugnani D, Airo P, Graf D, et al: Ineffective expression of CD40 ligand on cord blood T cells may contribute to poor immunoglobulin production in the newborn. *Eur J Immunol* 24:1919-1924, 1994
16. Cairo M: Neonatal neutrophil host defense. *Am J Dis Child* 143:40, 1989
17. Cairo M, Suen Y, Knoppel E, et al: Decreased G-CSF and IL-3 production and genetic expression from mononuclear cells of newborn infants. *Pediatr Res* 31:574, 1992

18. Cairo M, Suen Y, Knoppel E, et al: Decreased stimulated GM-CSF production and GM-CSF gene expression but normal numbers of GM-CSF receptors in human term newborns compared to adults. *Pediatr Res* 30:362, 1991
19. Campana D, Janosy G, Coustain-Smith E, et al: The expression of T cell receptor associated proteins during T cell ontogeny in man. *J Immunol* 142:57, 1989
20. Carr R, Modi N: Haemopoietic colony stimulating factors for preterm neonates. *Arch Dis Child* 76:F128-133, 1997
21. Casali P, Burastero S, Nakamura M, et al: Human lymphocytes making rheumatoid factor and antibody to ssDNA belong to Leu-1 + B-cell subset. *Science* 236:77-80, 1987
22. Casali P, Notkins AL: CD5 + B lymphocytes, polyreactive antibodies and the human B cell repertoire. *Immunol Today* 10:362-368, 1989
23. Cates K, Rowe J, Ballow M: The premature infant as a compromised host. *Curr Probl Pediatr* 13:1-63, 1983
24. Cates KL, Goetz C, Rosenberg N, et al: Longitudinal development of specific and functional antibody in very low birth weight premature infants. *Pediatr Res* 23:14-23, 1988
25. Chang M, Suen Y, Lee SM, et al: Transforming growth factor- β 1 macrophage inflammatory protein-1 α and interleukin-8 gene expression is lower in stimulated human neonatal compared with adult mononuclear cells. *Blood* 84:118-124, 1994
26. Chawareewong S, Jirapongsa A, Lokaphadhana K: Immune response to hepatitis B vaccine in premature neonates. *Southeast Asian J Trop Med Public Health* 22:39-40, 1991
27. Chheda S, Palkowetz K, Garogalo R, et al: Decreased interleukin-10 production by neonatal monocytes and T cells: Relationship to decreased production and expression of tumor necrosis factor- α and its receptors. *Pediatr Res* 40:475-483, 1996
28. Chin T, Ank B, Murakami D, et al: Cytotoxic studies in human newborns: Lessened allogeneic cell-induced (augmented) cytotoxicity but strong lymphokine-activated cytotoxicity of cord mononuclear cells. *Cell Immunol* 103:241-251, 1986
29. Choi Y, Rickert MH, Ballow M, et al: Human IgH-V gene repertoire in neonatal cord blood, adult peripheral blood, and EBV-transformed cells. *Ann NY Acad Sci* 764:261-264, 1995
30. Clark EA, Ledbetter JA: How B and T cells talk to each other. *Nature* 367:425-428, 1994
31. Clark S, Kamen R: The human hematopoietic colony-stimulating factors. *Science* 236:1229-1237, 1987
32. Clement LT: Isoforms of the CD45 common leukocyte antigen family: Markers for human T-cell differentiation. *J Clin Immunol* 12:1, 1992
33. Clerici M, DePalma L, Roilides E, et al: Analysis of T helper and antigen-presenting cell functions in cord blood and peripheral blood leukocytes from healthy children of different ages. *J Clin Invest* 91:2829-2836, 1993
34. Conley ME, Larche M, Bonagura VR, et al: Hyper IgM syndrome associated with defective CD40-mediated B cell activation. *J Clin Invest* 94:1404-1409, 1994
35. Cooper M, Buckley R: Developmental immunology and the immunodeficiency diseases. *JAMA* 248:2658-2670, 1982
36. Dancis J, Osborn JJ, Kunz HW: Studies of immunology of the newborn infant, IV: Antibody formation in the premature infant. *Pediatrics* 12:151-157, 1953
37. deBont E, Martens A, vanRaaij J, et al: Tumor necrosis factor- α , interleukin-1 β , and interleukin-6 plasma levels in neonatal sepsis. *Pediatr Res* 33:380-383, 1993
38. Durandy A, Hivroz C, Mazerolles F, et al: Abnormal CD40-mediated activation pathway in B lymphocytes from patients with hyper-IgM syndrome and normal CD40 ligand expression. *J Immunol* 158:2576-2584, 1997
39. Durandy A, Thuillier L, Forveille M, et al: Phenotypic and functional characteristics of human newborns' B lymphocytes. *J Immunol* 144:60-65, 1990
40. Eichenwald E: Perinatally transmitted neonatal bacterial infections. *Infect Dis Clin North Am* 11:223, 1997
41. Einhorn MS, Granoff DM, Nahm MH, et al: Concentrations of antibodies in paired maternal and infant sera: Relationships to IgG subclass. *J Pediatr* 111:783-788, 1987
42. Elsasser-Beile U, Dursunoglu B, Gallati H, et al: Comparison of cytokine production

- in blood cell cultures of health children and adults. *Pediatr Allergy Immunol* 6:170-174, 1995
43. English BK, Burchett SK, English JD, et al: Production of lymphotoxin and tumor necrosis factor by human neonatal mononuclear cells. *Pediatr Res* 24:717, 1988
 44. Erkeller-Yuksel FM, Deney V, Yuksel B, et al: Age-related changes in human blood lymphocyte subpopulations. *J Pediatr* 1120:216-222, 1992
 45. European Collaborative Study: Age-related standards for T lymphocyte subsets based on uninfected children born to human immunodeficiency virus 1-infected women. *Pediatr Infect Dis* 11:1018-1026, 1992
 46. Evans HE, Akpata SO, Glass L: Serum immunoglobulin levels in premature and full-term infants. *Am J Pathol* 56:416-418, 1971
 47. Ferreira AA, Bunn-Moreno M, Sant Anna C, et al: BCG vaccination in low birth weight newborns: Analysis of lymphocyte proliferation, IL-2 generation of intradermal reaction to PPD. *Tubercle and Lung Disease* 77:476-481, 1996
 48. Ferrieri P: Neonatal susceptibility and immunity to major bacterial pathogens. *Reviews of Infectious Diseases* 12:S392, 1990
 49. Fowlkes BJ, Pardoll DM: Molecular and cellular events of T cell development. *Adv Immunol* 44:207-264, 1989
 50. Gaddy J, Risdon G, Broxmeyer HE: Cord blood natural killer cells are functionally and phenotypically immature but readily respond to interleukin-2 and interleukin-12. *J Interferon Cytokine Res* 15:527, 1995
 51. Gandhi A, Brodsky L, Ballow M: Benefits of antibiotic prophylaxis in children with chronic sinusitis: Assessment of outcome predictors. *Allergy Proc* 14:37-43, 1993
 52. Garderet L, Dulphy N, Douay C, et al: The umbilical cord blood $\alpha\beta$ T-cell repertoire: Characteristics of a polyclonal and naive but completely formed repertoire. *Blood* 91:340-346, 1998
 53. Gathings WE, Lawton AR, Cooper MD: Immunofluorescent studies of the development of pre-B cells, B lymphocytes and immunoglobulin isotype diversity in humans. *Eur J Immunol* 7:804, 1977
 54. George JFJ, Schroeder HWJ: Developmental regulation of D β reading frame and junctional diversity in T cell receptor- β transcripts from human thymus. *J Immunol* 148:1230-1239, 1992
 55. Gerli R, Agea E, Muscat C, et al: Activation of cord T lymphocytes. *Cell Immunol* 148:32-47, 1993
 56. Gold MR, de Franco AL: Biochemistry of B lymphocyte activation. *Adv Immunol* 55:221-295, 1994
 57. Goodnow CC: Transgenic mice and analysis of B cell tolerance. *Annu Rev Immunol* 10:489-518, 1992
 58. Granoff DM, Shackelford PG, Pandey JP, et al: Antibody responses to *Haemophilus influenzae* type b polysaccharide vaccine in relation to Km (1) and G2m (23) immunoglobulin allotypes. *J Infect Dis* 154:257-264, 1986
 59. Hassan J, Reen D: Neonatal CD4, CD45RA, T cells: Precursors of adult CD4, CD45RA T cells. *Res Immunol* 144:87-92, 1993
 60. Haynes BF, Heinly CS: Early human T cell development: Analysis of the human thymus at the time of initial entry of hematopoietic stem cells into the fetal thymic microenvironment. *J Exp Med* 181:1445, 1995
 61. Haynes BF, Martin ME, Kay HH, et al: Early events in human T cell ontogeny. Phenotypic characterization and immunohistologic localization of T cell precursors in early human fetal tissues. *J Exp Med* 168:1061-1080, 1988
 62. Hayward A, Cosyns M: Proliferative and cytokine responses by human newborn T cells stimulated with staphylococcal enterotoxin B. *Pediatr Res* 35:293-298, 1994
 63. Hayward A, Groothuis J: Development of T cells with memory phenotype in infancy. In Mestecky J (ed): *Immunology of Milk and the Neonate*. New York, Plenum Press, 1991, p 71
 64. Hickey S, McCracken G: Postnatal bacterial infections. In Fanaroff AA, Martin RJ (eds): *Neonatal-Perinatal Medicine: Diseases of the Fetus and Infant*, ed 6. St. Louis, Mosby-Year Book, 1997, pp 717-720
 65. Hill H: Biochemical, structural, and functional abnormalities of polymorphonuclear leukocytes in the neonate. *Pediatr Res* 22:375, 1987

66. Hobbs JR, Davis JA: Serum G-globulin levels and gestational age in premature babies. *Lancet* 1:77, 1967
67. Hohlfield P, Forestier F, Marion S, et al: *Toxoplasma gondii* infection during pregnancy T lymphocyte subpopulation in mothers and fetuses. *Pediatr Infect Dis J* 9:878-881, 1990
68. Holtmeier W, Witthoft T, Hennemann A, et al: The TCR- δ repertoire in human intestine undergoes characteristic changes during fetal to adult development. *J Immunol* 158:5632-5461, 1997
69. Horst E, Meijer CJLM, Duijvestijn AM, et al: The ontogeny of human lymphocyte recirculation: High endothelial cell antigen (HECA-452) and CD44 homing receptor expression in the development of the immune system. *Eur J Immunol* 20:1483-1489, 1990
70. Hoshino T, Yamada A, Honda J, et al: Tissue-specific distribution and age-dependent increase of human CD11b T cells. *J Immunol* 151:2237-2246, 1993
71. Hyvarinen M, Zelter P, Oh W: Influence of gestational age on serum levels of alpha-1 fetoprotein, IgG globulin, and albumin in newborn infants. *J Pediatr* 82:430-437, 1973
72. Itohara S, Mombaerts P, Lafaille J, et al: T cell receptor δ gene mutant mice: Independent generation of $\alpha\beta$ T cells and programmed rearrangements of $\gamma\delta$ TCR genes. *Cell* 72:337-348, 1993
73. Jameson SC, Hogquist KA, Bevan MJ: Positive selection of thymocytes. *Annu Rev Immunol* 13:93-126, 1996
74. Kagi D, Ledermann B, Burki K, et al: Molecular mechanisms of lymphocyte-mediated cytotoxicity and their role in immunological protection and pathogenesis in vivo. *Annu Rev Immunol* 14:207-232, 1996
75. Kantor: The development and repertoire of B-1 cells (CD5 cells). *Immunol Today* 12:389-391, 1991
76. Kappler JW, Roehm M, Marack PT: T cell tolerance by clonal selection in the thymus. *Cell* 49:273-280, 1987
77. Kawano Y, Noma T: Role of interleukin-2 and interferon- γ in inducing production of IgG subclasses in lymphocytes of human newborns. *Immunology* 88:40-48, 1996
78. Kibler R, Hicks MJ, Wright AL, et al: A comparative analysis of cord blood and adult lymphocytes: Interleukin-2 and interferon production, natural killer cell activity, and lymphocyte populations. *Diagnostic Immunology* 4:201-208, 1986
79. Kim C, Pelus L, White J, et al: Differential chemotactic behavior of developing T cells in response to thymic chemokines. *Blood* 91:4434-4443, 1998
80. Klein J, Marcy S: Bacterial sepsis and meningitis. In Remington J, Klein J (eds): Infectious diseases of the fetus and newborn infant, ed 4. Philadelphia, WB Saunders, 1995, pp 836-878
81. Kohler PF, Farr RS: Elevation of cord over maternal IgG immunoglobulin: Evidence for an active placental IgG transport. *Nature* 210:1070-1071, 1966
82. Kotiranta-Ainamo A, Rautonen J, Rautonen N: Interleukin-10 production by cord blood mononuclear cells. *Pediatr Res* 41:110-113, 1997
83. Krediet TG, Beurskens FJM, van Duk H, et al: Antibody responses and opsonic activity in sera of preterm neonates with coagulase-negative staphylococcal septicemia and the effect of the administration of fresh frozen plasma. *Pediatr Res* 43:645-651, 1998
84. Lau YL, Tam AYC, Ng KW, et al: Response of preterm infants to hepatitis B vaccine. *J Pediatr* 121:962-965, 1992
85. Lee S, Knoppel E, van de Ven C, et al: Transcriptional rates of granulocyte-macrophage colony-stimulating factor, granulocyte colony stimulating factor, interleukin-3 and macrophage colony-stimulating factor genes in activated cord versus adult mononuclear cells: Alteration in cytokine expression may be secondary to post-transcriptional instability. *Pediatr Res* 34:560, 1993
86. Leider JM: Transcriptional regulation of T cell receptor genes. *Annu Rev Immunol* 11:539-570, 1993
87. Leino A, Ruuskanen O, Kero P, et al: Depressed phytohemagglutinin and concanavalin A responses in premature infants. *Clin Immunol Immunopathol* 19:260-267, 1981
88. Lewis DB: Cellular immunity of the human fetus and neonate. *Immunology and Allergy Clinics of North America* 18:291, 1998

89. Lewis DB, Yu CC, Meyer J, et al: Cellular and molecular mechanisms for reduced interleukin 4 and interferon- γ production by neonatal T cells. *J Clin Invest* 87:194–202, 1991
90. Liechty K, Koenig J, Mitchell M, et al: Production of interleukin-6 by fetal and maternal cells in vivo during intraamniotic infection and in vitro after stimulation with interleukin-1. *Pediatr Res* 29:1, 1991
91. Lilic D, Cant A, Abinun M, et al: Cytokine production differs in children and adults. *Pediatr Res* 42:237–240, 1997
92. Liu CC, Young LHY, Young JDE: Lymphocyte-mediated cytolysis and disease. *New Engl J Med* 335:1651–1659, 1996
93. Liu YJ, Malisan F, deBouteiller O, et al: Within germinal centers, isotype switching of immunoglobulin genes occurs after the onset of somatic mutation. *Immunity* 4:241–250, 1996
94. Lubens R, Gard S, Soderberg-Watner M, et al: Lectin-dependent T lymphocyte and natural killer cytotoxic deficiencies in human newborns. *Cell Immunol* 74:40–53, 1982
95. Lucivero G, Dell'Osso A, Iannone A, et al: Phenotypic immaturity of T and B lymphocytes in cord blood of full-term normal neonates. *Biol Neonate* 44:303–308, 1983
96. Marshall LS, Aruffo A, Ledbetter JA, et al: The molecular basis for T cell help in humoral immunity: CD40 and its ligand, gp39. *J Clin Immunol* 13:165–174, 1993
97. Matsuda F, Honjo T: Organization of the human heavy chain locus. *Adv Immunol* 62:1–29, 1996
98. McNabb T, Koh TY, Dorrington KJ, et al: Structure and function of immunoglobulin domains. V. Binding of immunoglobulin G and fragments to placental membrane preparations. *J Immunol* 117:882–888, 1976
99. Merrill JD, Sigaroudinia M, Kohl S: Characterization of natural killer and antibody-dependent cellular cytotoxicity of preterm infants against human immunodeficiency virus-infected cells. *Pediatr Res* 40:498, 1996
100. Michie CA, McLean A, Alcock C, et al: Lifespan of human lymphocyte subsets defined by CD45 isoforms. *Nature* 360:264–265, 1992
101. Miyawake T, Seki H, Taga K, et al: Disassociated production of interleukin-2 and immune (γ) interferon by phytohaemagglutinin-stimulated lymphocytes in healthy infants. *Clin Exp Immunol* 59:505–511, 1985
102. Montagna D, Moretta A, Marconi M, et al: In vivo activated cord blood lymphocytes express high affinity interleukin-2 receptor: Evaluation of their responsiveness to in vitro stimulation with recombinant interleukin-2. *Biol Neonate* 62:385–394, 1992
103. Morita CT, Parker C, Brenner MB, et al: TCR usage and functional capabilities of human $\gamma\delta$ T cells at birth. *J Immunology* 153:3979, 1994
104. Muller K, Zak M, Nielsen S, et al: In vitro cytokine production and phenotype expression by blood mononuclear cells from umbilical cords, children and adults. *Pediatr Allergy Immunol* 7:117–124, 1996
105. Nonoyama S, Penix LA, Edwards CP, et al: Diminished expression of CD40 ligand by activated neonatal T cells. *J Clin Invest* 96:66, 1995
106. Ochs HD, Wedgewood RJ: IgG subclass deficiencies. *Annu Rev Med* 38:325–340, 1987
107. Ozdemir A, Oygur N, Gultikin M, et al: Neonatal tumor necrosis factor, interleukin-1, interleukin-1B and interleukin-6 response to infection. *Am J Perinatol* 11:282–285, 1994
108. Pabst HF, Godel JC, Spady DW, et al: Transfer of maternal specific cell-mediated immunity to the fetus. *Clin Exp Immunol* 68:209–214, 1987
109. Panaro A, Amati A, diLoreto M, et al: Lymphocyte subpopulations in pediatric age. Definition of reference values by flow cytometry. *Allergol Immunopathol (Madr)* 19:109–112, 1991
110. Peakman M, Buggins AG, Nicolaides KH, et al: Analysis of lymphocyte phenotypes in cord blood from early gestation fetuses. *Clin Exp Immunol* 90:345, 1992
111. Perlmutter RM, Kearney JF, Chang SP: Developmentally controlled expression of immunoglobulin VH genes. *Science* 227:1597–1601, 1985
112. Phillips J, Hori T, Nagler A, et al: Ontogeny of human natural killer (NK) cells: Fetal NK cells mediate cytolytic function and express cytoplasmic CD3 ϵ , δ proteins. *J Exp Med* 175:1055–1066, 1992

113. Piccinni MP, Beloni L, Giannarini L, et al: Abnormal production of T helper 2 cytokines interleukin-4 and interleukin-5 by T cells from newborns with atopic parents. *Eur J Immunol* 26:2293-2298, 1996
114. Pirenne H, Aujard Y, Eljaafari A, et al: Comparison of T cell functional changes during childhood with the ontogeny of CDw29 and CD45RA expression on CD4+ T cells. *Pediatr Res* 32:81-86, 1992
115. Pirenne-Ansart H, Paillard F, De Groote D, et al: Defective cytokine expression but adult-type T-cell receptors, CD8, and p56 modulation in CD3 or CD2 activated T cells from neonates. *Pediatr Res* 37:64-69, 1995
116. Qian JX, Lee SM, Suen Y, et al: Decreased interleukin-15 from activated cord versus adult peripheral blood mononuclear cells and the effect of interleukin-15 in upregulating antitumor immune activity and cytokine production in cord blood. *Blood* 90:3106-3117, 1997
117. Raaphorst FM, Timmers E, Kenter MJ, et al: Restricted utilization of germ-line VH3 genes and short diverse third complementarity-determining regions (CDR3) in human fetal B lymphocyte immunoglobulin heavy chain rearrangements. *Eur J Immunol* 22:247, 1992
118. Rainaut M, Pagniez M, Hercent T, et al: Characterization of mononuclear cell subpopulations in normal fetal peripheral blood. *Human Immunology* 18:331-337, 1987
119. Raveche ES: Possible immunoregulatory role for CD5+ B cells. *Clin Immunol Immunopathol* 56:135-150, 1990
120. Reth M: Antigen receptors on B lymphocytes. *Annu Rev Immunol* 10:97-121, 1992
121. Ridings J, Nicholson IC, Goldsworthy W, et al: Somatic hypermutation of immunoglobulin genes in human neonates. *Clin Exp Immunol* 108:366-374, 1997
122. Risdon G, Gaddy J, Stehman F, et al: Proliferative and cytotoxic responses of human cord blood T lymphocytes following allogenic stimulation. *Cell Immunol* 154:14-24, 1994
123. Rothenberg E, Triglia D: Clonal proliferation unlinked to terminal deoxynucleotidyl transferase synthesis in thymocytes of young mice. *J Immunol* 130:1627, 1983
124. Sanders ME, Makgoba MW, Shaw S: Human naive and memory T cells: Reinterpretation of helper-inducer and suppressor-inducer subsets. *Immunol Today* 9:195-199, 1988
125. Sautois B, Fillet G, Beguin Y: Comparative cytokine production by in vitro stimulated mononucleated cells from cord blood and adult blood. *Experimental Hematology* 25:103-108, 1997
126. Schatz DG, Oettinger MA, Schlissel MS: V(D)J recombination: Molecular biology and regulation. *Annu Rev Immunol* 10:359-383, 1992
127. Schelonka R, Raaphorst F, Infante D, et al: T cell receptor repertoire diversity and clonal expansion in human neonates. *Pediatr Res* 43:396, 1998
128. Schibler K, Liechty K, White W, et al: Defective production of interleukin-6 by monocytes: A possible mechanism underlying several host defense deficiencies of neonates. *Pediatr Res* 31:18-21, 1992
129. Schroeder HW, Hillson JL, Perlmutter RM: Early restriction of the human antibody repertoire. *Science* 238:791-793, 1987
130. Schroeder HW, Perlmutter RM: Development of the human antibody repertoire. In Griscelli C, Gupta S (eds): *New Concepts in Immunodeficiency Diseases*. London, John Wiley and Sons, 1993, pp 1-20
131. Schroeder HWJ, Mortari F, Shiokawa S, et al: Development regulation of the human antibody repertoire. *Ann NY Acad Sci* 764:242-260, 1995
132. Seghaye M, Heyl W, Grabitz R, et al: Production of pro and anti inflammatory cytokines in neonates assessed by stimulated whole cord blood cultures and by plasma levels at birth. *Biol Neonates* 73:220-227, 1998
133. Series I, Pichette J, Carrier C, et al: Quantitative analysis of T/B cell subsets in healthy and sick premature infants. *Early Hum Dev* 26:143-154, 1991
134. Sieff C: Hematopoietic growth factors. *J Clin Invest* 79:1549-1557, 1987
135. Sloan-Lancaster J, Allen PM: Altered peptide ligand-induced partial T-cell activation: Molecular mechanisms and role in T cell biology. *Annu Rev Immunol* 14:1-27, 1996
136. Smith RT, Eitzman DV, Catlin ME: The development of the immune response: Charac-

- terization of the response of the human infant and adult to immunization with *Salmonella* vaccines. *Pediatrics* 33:163-183, 1964
137. Spits H, Lanier L, Phillips J: Development of human T and natural killer cells. *Blood* 85:2654-2670, 1995
 138. Splawski J, Lipsky P: Cytokine regulation of immunoglobulin secretion by neonatal lymphocytes. *J Clin Invest* 88:967-977, 1991
 139. Splawski JB, Jelinek DF, Lipsky PE: Delineation of the functional capacity of human neonatal lymphocytes. *J Clin Invest* 87:545, 1991
 140. Stites D, Pavia C: Ontogeny of human T cells. *Pediatrics* 64:795-802, 1979
 141. Stoll B: The global impact of neonatal infection. *Clin Perinatol* 24:1, 1997
 142. Strominger J: Developmental biology of T cell receptors. *Science* 24:943, 1989
 143. Stutman O: Ontogeny of T Cells. *Clinics Immunol Allergy* 5:191-234, 1985
 144. Suen Y, Lee S, Cairo M: Decreased M-CSF mRNA expression from activated cord versus adult mononuclear cells: Altered post transcriptional stability. *Blood* 84:4269, 1994
 145. Takahashi N, Imanishi K, Nishida H, et al: Evidence for immunologic immaturity of cord blood T cells. *J Immunol* 155:5213-5219, 1995
 146. Tang M, Kemp A: Ontogeny of IL-4 production. *Pediatric Allergy and Immunology* 6:11-19, 1995
 147. Thomas R, Linch D: Identification of lymphocyte subsets in the newborn using a variety of monoclonal antibodies. *Arch Dis Child* 58:34-38, 1983
 148. Toivanen P, Uksila J, Leino A, et al: Development of mitogen responding T cells and natural killer cells in the human fetus. *Immunol Rev* 57:89-105, 1981
 149. Ugazio AG, Marcioni AF, Astaldi A, et al: Peripheral blood B lymphocytes in infancy and childhood. *Acta Paediatr Scand* 63:205-208, 1974
 150. Vahlquist B, Nordbring F: Studies on diphtheria, IV: The effects of diphtheria immunization on newborn prematures. *Acta Paediatr* 41:53-56, 1952
 151. Vitiello A, Maccario R, Montagna D, et al: Lymphocyte subpopulations in the Neonate: A subset of HNK-1, OKT3, OKT8, lymphocytes displays natural killer activity. *Cell Immunol* 85:252-257, 1984
 152. Vogler LB, Lawton AR: Ontogeny of B cells and humoral immune functions. *Immunology and Allergy Clinics of North America* 5:235-252, 1985
 153. Wilson C, Westall J, Johnston L, et al: Decreased production of interferon-gamma by human neonatal cells. *J Clin Invest* 77:860-867, 1986
 154. Wilson M: Immunology of the fetus and newborn: Lymphocyte phenotype and function. *Immunology and Allergy Clinics of North America* 5:271-286, 1985
 155. Wilson M, Rosen FS, Schlossman SF, et al: Ontogeny of human T and B lymphocytes during stressed and normal gestation: Phenotypic analysis of umbilical cord lymphocytes from term and preterm infants. *Clin Immunol Immunopathol* 37:1, 1985
 156. Wolach B: Neonatal sepsis: Pathogenesis and supportive therapy. *Semin Perinatol* 21:28-38, 1997
 157. Yachie A, Takano N, Yokoi T, et al: The capability of neonatal leukocytes to produce IL-6 on stimulation assessed by whole blood culture. *Pediatr Res* 27:227-233, 1990
 158. Yang L, Demeure C, Byun D, et al: Maturation of neonatal human CD4 T cells, III: Role of B7 co-stimulation at priming. *Int Immunol* 7:1987-1993, 1995
 159. Yeung CY, Hobbs JR: Serum-gamma G-globulin levels in normal, premature, post-nature, and "small-for-dates" newborn babies. *Lancet* 1:1167-1170, 1968
 160. Zola H, Fusco M, Weedon H, et al: Reduced expression of the interleukin-2 receptor- γ chain on cord blood lymphocytes: Relationship to functional immaturity of the neonatal immune response. *Immunology* 87:86-91, 1996

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