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Immunology of Pediatric HIV Infection

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Summary

Most infants born to human immunodeficiency virus (HIV)-infected women escape HIV infection. Infants evade infection despite an immature immune system and, in the case of breastfeeding, prolonged repetitive, exposure. If infants become infected, the course of their infection and response to treatment differs dramatically depending upon the timing (*in utero*, intrapartum, or during breastfeeding) and potentially the route of their infection. Perinatally acquired HIV infection occurs during a critical window of immune development. HIV's perturbation of this dynamic process may account for the striking age-dependent differences in HIV disease progression. HIV infection also profoundly disrupts the maternal immune system upon which infants rely for protection and immune instruction. Therefore, it is not surprising that infants who escape HIV infection still suffer adverse effects. In this review, we highlight the unique aspects of pediatric HIV transmission and pathogenesis with a focus on mechanisms by which HIV infection during immune ontogeny may allow discovery of key elements for protection and control from HIV.

Keywords

HIV; children; neonatal; gut microbiota; breast milk; Th17; regulatory T cells

Introduction

Most children born to human immunodeficiency virus-1 (HIV)-infected women do not become infected. Infants evade infection despite an immature immune system and, in the case of breastfeeding prolonged repetitive, exposure. If infants become infected, the course of their infection and response to treatment differs dramatically depending upon the timing and potentially the route of their infection. Perinatally acquired HIV infection occurs during a critical window of immune development. HIV's perturbation of this dynamic process may account for the striking age-dependent differences in HIV disease progression. HIV infection also profoundly disrupts the maternal immune system upon which infants rely for protection and immune instruction. Therefore, it is not surprising that infants who escape HIV infection still suffer adverse effects. In this review, we highlight the unique aspects of pediatric HIV transmission and pathogenesis with a focus on mechanisms by which HIV infection during immune ontogeny may allow discovery of key elements for protection and control from HIV.

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Inefficiency of HIV transmission

The majority of pediatric HIV infection occurs via mother-to-child transmission (MTCT) at three distinct time points: *in utero*, intrapartum, or through breast milk. In chronically HIV-infected women not receiving antiretroviral therapy (ART), transmission rates are approximately 5–10%, 10–20%, and 5–15%, respectively (1). Maternal levels of HIV are a major risk factor in all forms of transmission, while a rare polymorphism in the chemokine receptor CCR5 is the most protective. Nevertheless, most children born to women with high levels of HIV and low numbers of $CD4^+$ T cells do not become infected. Even when primary HIV infection occurs during pregnancy and levels of viremia are extremely high, 80% of children escape infection (2). In addition, some children elude *in utero* and intrapartum infection but later acquire HIV through breastfeeding. These observations suggest that other phenomena modify the interplay between maternal infectiousness and infant susceptibility. Biologic, behavioral, and environmental factors associated with transmission are summarized in Table 1. Despite decades of research, the role of immunity versus viral exposure (quantity and quality) in transmission remains elusive and controversial (3). Below we briefly review the biology of MTCT.

In utero transmission

Transplacental HIV transmission is the least efficient form of MTCT. Despite 9 months of exposure at the maternofetal interface and evidence of bi-directional cellular trafficking between mother and fetus, only 5–10% of children become infected (4, 5). Variable but persistent levels of maternal cells can be detected in healthy children, suggesting that significant numbers of infants are exposed to HIV-1 *in utero*. Indeed, maternal cells can be detected in the blood of HIV-uninfected infants born to HIV-infected women (5, 6). Why then are most fetuses not infected?

A compelling hypothesis is that the developmentally regulated propensity of fetal cells to avoid inflammatory responses thwarts infection. Most fetal cells are quiescent, while efficient productive HIV infection only occurs in activated T cells (7). The fetomaternal cellular exchanges that potentially transmit HIV also promote the development of fetal T-regulatory cells (Tregs). These Treg cells actively suppress immune responses to maternal antigens (8). *In utero* exposure to foreign antigens, e.g. placental malarial infection, has been associated with increased Treg cell populations at birth (9). The observation that *in vitro* depletion of Treg cells in HIV-1-exposed uninfected infants increases the detection of HIV-specific responses is consistent with this hypothesis (10). Further support is provided by studies demonstrating the strong associations between *in utero* transmission and placental inflammation (11–14).

Although immune quiescence is an attractive hypothesis, the human fetal intestine is populated with the natural targets of HIV infection ($CD4^+CCR5^+$ T cells), and *in vitro*, these cells are susceptible to HIV infection (15). Additionally, *ex vivo* fetal enterocytes have a marked inflammatory propensity when stimulated and this persists until term (see below). In fact, fetal tissues and cells are more permissive to HIV infection compared to adult cells and tissues (16–19). This observation raises the possibility that additional amniotic and/or placental factors quell fetal inflammatory intestinal responses *in utero*. Innate and adaptive placental cellular responses with immunomodulatory and/or antiviral properties have been described (20–24), and whether these or other factors play a role is uncertain.

Intrapartum transmission

In the absence of antiretroviral (ARV) prophylaxis, the majority of HIV-transmission occurs at the time of delivery. Whether this is due to transplacental transfer of virus during

parturition or infant exposure to maternal secretions and blood at delivery is uncertain. However, the protective effect of cesarean section (25) coupled with the presence of both HIV in infant gastric aspirates (26) and $CD4^+CCR5^+$ T cells in the neonatal gut (15) suggest most intrapartum transmission is likely mucosal. Additionally, the inflammatory cytokines and chemokines present in placentas of mothers who transmit *in utero* are not seen in the placentas of mothers who transmit during delivery, suggesting a different mechanism of transmission that is unlikely to be transplacental (11). Factors that increase the risk of likelihood of intrapartum transmission are listed in Table 1.

A unique feature of pediatric transmission is that it generally occurs in the presence of HIV-specific antibody. During the third trimester of gestation, maternal immunoglobulin G (IgG) antibodies are transplacentally transferred, such that at term, infant levels are equivalent to or higher than those in the maternal circulation (27). These antibodies are associated with infant protection against a variety of microbes (28). Although maternal antibodies have been associated with inhibition of infant immune responses, this suppressive effect is highly variable and does not preclude the generation of T-cell responses and/or priming (29). A key remaining question is whether maternal antibodies (neutralizing and/or non-neutralizing) modify transmission risk and/or disease progression in HIV-infected infants. Studies on the role of maternal antibodies in MTCT are inconsistent, but in simian immunodeficiency virus (SIV) models, broadly neutralizing antibodies can prevent infection (30–34) and modify immunity in infected neonatal macaques (32). These studies provide proof of principle for the use of broad-neutralizing monoclonal antibodies in preventing MTCT in high-risk infants.

Breast milk transmission

Fewer than one in five infants become infected via breast milk, even though the immune system is not yet ‘fully mature’ and the infants are exposed up to every 2 hours for months to years (estimates include exposure to >60,000 infected cells and 500,000 cell-free virions daily) (35). Breast milk HIV-1 transmission, like sexual transmission, depends on (i) breaching an epithelial barrier that limits the amount of HIV in the transmitting fluid, (ii) remaining in an infectious form within the secretion, and (iii) traversing another mucosal surface to infect a new host.

The mammary epithelium is very effective in curtailing HIV entry into breast milk and most likely represents an important mechanism by which infant transmission is restricted. HIV RNA levels in breast milk are at least 100-fold lower than those in the circulation (36). In the absence of ART, approximately a third of HIV-infected women have breast milk HIV RNA levels below 50 copies/ml, while another third shed virus only intermittently (37). Breast milk viral shedding is increased by conditions associated with increased mammary epithelial permeability including parity, sudden changes in the frequency of breastfeeding, mastitis and abscess (38) with a concomitant increase in transmission (39, 40). However, even in the setting of a breast abscess, breast milk HIV levels remain significantly below levels in the bloodstream (41). Interestingly, this viral restriction is quantitative not qualitative. The HIV variants that enter breast milk are genetically indistinguishable from those in contemporaneous plasma (36, 42–44). Within breast milk there is limited viral evolution, which may reflect restricted replication (36, 43, 45). This is a surprising finding given the marked immunologic differences between the breast milk and blood (46) but suggests that the genetic bottleneck observed during transmission does not occur in breast milk. The factors governing viral restriction are incompletely understood; however, there is increasing evidence demonstrating that epithelial cells have dynamic and reciprocal interactions with adaptive and innate immune factors (47).

Breastfeeding behavior also plays an important role in transmission risk. Exclusive breastfeeding reduces HIV breast milk transmission by more than 50% compared to mixed feeding, i.e. feeding infants water, other liquids, and/or solids in addition to breast milk (48). This observation seems counterintuitive as exclusively breastfed infants have greater HIV exposure, yet the findings are reproduced in several large studies including two set out specifically to examine this hypothesis (49–53). Although it has long been recognized that exclusive breastfeeding is associated with decreased morbidity and mortality in non-HIV infected populations (54–56), why this simple behavior would so consistently and dramatically prevent HIV infection remains a 'milk mystery'. Two non-mutually exclusive mechanisms have been proposed. The first is that mixed feeding disrupts the gastrointestinal epithelium by (i) introducing dietary antigens and/or pathogens, (ii) decreasing the exposure to the bioactive molecules in milk that promote intestinal integrity, decrease inflammation, and provide anti-microbial protection, and/or (iii) displacing or altering commensal microbial communities. The second potential mechanism is that non-exclusive breastfeeding disrupts mammary epithelia integrity. Women produce milk at a rate largely dictated by infant suckling. Interruptions and/or displacements of infant feeding disrupt the natural homeostasis reached between milk production and milk ejection in the mother-infant pair. This disruption leads to milk stasis and breast engorgement with subsequent increases in mammary epithelial tight junctions. If milk is not ejected, epithelial permeability increases, allowing the bidirectional passage of substances between the milk ducts and the circulation and produces a local and systemic inflammatory response (mastitis). Non-exclusive breastfeeding is associated with breast pathology, whereas exclusive breastfeeding reduces mastitis and breast abscess, both of which are associated with increased viral load (57). Whether the increase in transmission with non-exclusive breastfeeding is due to changes in breast milk viral load, the inflammatory response that accompanies changes in mammary epithelial integrity, the interruption of the constant immune modulation provided by breast milk, or the increase in foreign antigens as the infant gut is exposed to formula or a combination of these factors is unknown (57–61).

Breast milk contains a rich array of factors that could potentially facilitate or hinder HIV transmission either directly or indirectly (Table 2). To date, most studies have been limited by small sample size and a focus on a single or a few factors while protection is likely multi-factorial and may differ between cell free and cell-associated virus (62). Another confounding factor is that the composition of milk is highly variable between women and over time. Given the evolving nature of the suckling child's oral mucosa, intestinal tract and immune system, protection may be mediated by different factors at different times. Not surprisingly, high concentrations of pro-inflammatory molecules [e.g. CXCL12, CCL5 IL-8, RANTES, IL-8, IL-15, macrophage inflammatory protein 1a (MIP-1a)] have been associated with increased risk of transmission in most but not all studies, while low levels of breast milk IL-7 correlated with transmission in one study (63–69). Other breast milk components with protection in clinical studies include α-defensins (70, 71), long-chain n-6 polyunsaturated fatty acids (72), non 3′-sialyllactose (3′SL) human milk oligosaccharides (73), and erythropoietin (74). Although breast milk HIV-specific IgA was not associated with transmission (75), HIV-specific antibodies capable of antibody-dependent cell cytotoxicity (ADCC) were found to be protective in one study (76). Breast milk substances that interfere with HIV binding and infection *in vitro* include Lewis × factor, bile salt lipase, oligosaccharides, soluble Toll-like receptor 2, glycans, MUC1, and lactic acid bacteria (66, 67, 77–81).

Unlike other secretions, breast milk has a relatively high concentration of immune cells, including antigen-specific T cells directed towards pathogens unlikely to infect the breast, e.g. influenza and respiratory syncytial virus. This suggests that these cells play a role in infant protection either at mucosal surfaces or systemically (82–84). In rodents, these cells

home to the lactating gland from the maternal intestine and respiratory tract. In humans, these cells are phenotypically distinct from those in the blood (85–87). In animal studies, these cells can traverse the gastrointestinal tract and are functional in the suckling animal but human data are sparse and controversial (88–93). The role of breast milk immune cells in HIV transmission is unclear. HIV-specific $CD8^+$ T cells are enriched in the breast milk of HIV-infected women but do not correlate with HIV RNA levels in milk (84, 94); however, one study found an association with HIV-specific responses and peripartum HIV transmission (65). Activated HIV-specific breast milk B cells have also been described, but their role in transmission is unclear (95).

The most consistent predictors of HIV breast milk transmission are levels of HIV, breast pathology (inflammation), and non-exclusive breastfeeding. The mammary epithelium plays a very important role in curbing HIV transmission. A *pot pourri* of factors within breast milk have been associated with transmission risk, but the effects and sample size have been modest. Nevertheless, these factors alone or in combination appear to restrict HIV replication within breast milk and/or transmission. Comprehensive strategies and large sample sizes are needed to tease out which of these factors may confer protection from HIV.

Clinical course of pediatric HIV

Most *in utero* infections occur a few weeks prior to delivery (96), and the disease course in these infants is strikingly different from infants infected at the time of birth with a median survival time of 208 versus 380 days (97). Infants delivered prior to 34 weeks of gestation are twice as likely to become infected intrapartum or through breast milk than infants born after 37 weeks of gestation (term) (98). Infants infected peripartum are more than three times more likely to die 180 days post-infection than infants infected via breast milk (99) (Fig. 1). HIV-infected infants who switch to formula at 4 months of age rather than continue breastfeeding through 16 months die at a median of 8 months versus 17 months (40) (Fig. 2). These profound differences highlight the importance of timing and potentially the route of infection on both acquisition of infection and disease progression. Unfortunately, the biologic basis of these differences remains speculative.

The evolution of HIV infection in adults is slow with a 10- year median progression to AIDS or death. In the weeks following acute infection, plasma viremia decreases by 100 to 1,000-fold and is relatively stable at this ‘viral load set-point’ for many years. The plasma HIV RNA level and the absolute $CD4^+$ T-cell count at this set-point are independent predictors of disease progression. In contrast, during the first few months post-infection, infant plasma viremia generally increases 10-fold to levels that are much higher than those in adults (100, 101). Most untreated infants have plasma HIV RNA levels of >100,000 per ml, and levels of a million or greater per ml are not uncommon. In children, HIV RNA levels slowly decline and do not reach a set-point until around age 5 years (102)(Fig. 3).

Rates of clinical disease progression differ markedly between children and adults. Initial reports suggested there was a bimodal distribution: about 20–30% of children experienced rapid progression to AIDS or death in the first year of life, and the remainder of children deteriorated at rates similar to adults. However, this description oversimplifies the complexity of HIV infection in children. An analysis of almost 4,000 perinatally infected children revealed striking age-related differences in disease progression in children less than 5 years of age (103). Regardless of the level of $CD4^+$ T cells or plasma viremia, younger children are at greater risk of death or progression to AIDS compared to their older counterparts (Fig. 4). A 1-year-old child with 10% $CD4^+$ T cells has a 40% risk of progression to AIDS and 20% risk of death within 12 months. The risk for a 10-year-old with those same laboratory values is 7.4% for developing AIDS and 2.1% for dying within

12 months. The risk of progression to AIDS or death in a 1-year-old child with a plasma RNA level of 100,000 copies per ml is 11% and 5 % respectively, but a 10-year-old's risk is 5.1% and 2% respectively. After age 5 years, the risk of disease progression and death and the predictive value of $CD4^+$ T-cell and plasma RNA levels appear to be similar to those observed in young adults (103).

The reasons for these striking differences are unknown but are usually attributed to immunologic 'immaturity' and the rapid expansion of $CD4^+$ T cells that accompany somatic growth (see below). Other factors that have been suggested to contribute to the high level of viremia in young children include an increased susceptibility of infant cells to HIV-1 infection (104) and shared HLA alleles between mother and child, so that the virus is preselected for fitness in the infant (105, 106). Notably, children infected with HIV-1 during adolescence tend to fare better than adults (107) (Fig. 5).

Infant exposure occurs during immunological development

Normal development of the human immune system (Table 3)

Infants and young children are more susceptible to infection and have suboptimal responses to many vaccines. Recent studies suggest that differences between infant and adult immune responses are not due to an infant's inability to respond ('a baby immune system') but rather reflect age-specific patterns of response (28). In other words, infants are not incapable of responding to stimuli. Instead, their responses are qualitatively and quantitatively different. The infant emerges from a sterile environment where an untoward inflammatory response can lead to premature birth and/or death. *In utero*, the infant must actively tolerate self and her/his mother (108). Once born, the infant is assaulted by antigens and pathogens and must immediately learn how and when to respond. The transition from a bias towards tolerance to an effector or priming response occurs over several years. During these early vulnerable years the infant relies on maternal protection and 'instruction'. Mothers provide transplacental antibodies, the microbes that initially colonize the infant and breast milk. Breast milk contains thousands of immunomodulatory molecules that stimulate growth and guide the child's immune development such that dietary and non-invasive antigens are tolerated while pathogenic antigens are dispatched. This complex and dynamic fluid is synchronized with the infant's needs and provides important defenses against infection. These early maternal influences have profound life-long effects on the immune responses and propensity to develop disease. Although differences between adult and pediatric immunity are most striking in young children, full immune competence is not achieved until adolescence, and maturation is asynchronous between immune components. The major differences in pediatric and adult immunity are briefly reviewed and discussed in the context of HIV infection.

Compared to mice, humans are born with a relatively mature immune system. T cells populate the peripheral lymphoid tissues as early as 10 gestational weeks (109), and B lymphocytes and antigen-presenting cells (APCs) can be detected by the end of the first trimester of gestation (110). Generally neonatal immune cells including APCs (dendritic cells, monoctyes, and macrophages) do not respond to costimulation in the same manner as adult cells and possess a greater capacity to produce the anti-inflammatory cytokine interleukin-10 (IL-10), contributing to an immunologically permissive environment (108). The expression and function of Toll-like receptors (TLRs) are developmentally regulated and responses are modified by soluble circulating factors (111–116). In response to TLR stimulation, including lipopolysaccharide (LPS), infant cells produce lower levels of cytokines that support T-helper 1 (Th1) cell differentiation (IFN-γ and IL-12) and higher levels of cytokines that support Th17 cell differentiation (IL-6, IL-23 and IL-1beta) (115–118). These responses promote a tolerogenic immunologic milieu with a distinct Th2 and

Th17 bias. These propensities are due to distinct epigenetic profiles and processes as well as the differential expression of microRNAs regulating transcription of cytokine genes in fetal/neonatal cells compared to adult cells (119–121).

Adaptive immune responses are also highly age-dependent (122). The majority of neonatal T cells are naïve and exhibit diminished proliferative responses, and lower IL-2 and IFN-γ secretion. Many of these cells are recent thymic emigrants (RTE), which are hyperresponsive to IL-7 and relatively resistant to acquiring Th1 function (123). Maternal cells crossing the placenta induce Tregs. The Treg cells express the forkhead box p3 (Foxp3) transcription factor and constitute up to 15% of the $CD4^+$ T cells during fetal life and then decrease in the circulation(8). Most infant Tregs express the intestinal homing receptor $\alpha_4\beta_7$ and display preferential migration to the intestine (124). In contrast to other fetal and infant immune cells, the functional abilities of fetal/neonatal Tregs are equivalent to those observed in adults (125). Treg cells have a reciprocal relationship with Th17 cells and the balance between these two $CD3^+CD4^+$ T-cell subsets is believed to be critical for dictating pro- versus anti-inflammatory responses. In mice, mucosal Th17 cells depend on intestinal colonization with specific bacteria (126–129); however, the role of bacteria in human Th17 development is unknown. Human fetal intestines contain large numbers of Th17 cells (130), but the relative composition of Th17 and Treg in human infants and how this balance varies during development is unknown. Murine studies indicate that Treg cells, particularly those in the intestine are a heterogeneous population (131), that display tremendous functional plasticity especially under lymphopenic and inflammatory conditions (132). Studies of peripheral blood Th17 $CD4^+$ cells indicate this cell population is developmentally regulated and inversely related to age (133). Neonatal $CD4^+$ T cells also have reduced expression of CD40L and provide less help for B-cell function. Naive infant B cells express lower levels of CD21, CD40, CD80, CD86, and typically neonatal antibody responses are delayed in onset, achieve lower peak levels, and persist for a shorter period of time (134). Qualitatively these antibodies have less affinity maturation, heterogeneity, and differ in the distribution of IgG isotype (lower titers of IgG2) compared to adults (134).

In addition to these qualitative differences, there are marked age-related differences in the number of immune cells (Fig. 6). The absolute number and relative percentage of circulating $CD4^+$ T cells are much higher in infants and children (135, 136). Immediately after birth levels increase, peaking in the first few months of life at 3–4 times adult values and then slowly decline, reaching adult levels at around age 6 years (135) (Fig. 3). There is less variation in the number of $CD8^+$ T cells. Relative to their body size, the mass of the thymus is increased and thymopoiesis is much more active during childhood and begins involution after puberty (137).

Levels of transplacental maternal antibodies decrease over the first few months of life as infant B-cell responses mature (138) (Fig. 7). However, even after one year of age, Ig levels, particularly IgA, remain well below those in adults. IgG subclass production also matures slowly reaching 60% of adult levels for IgG1 and IgG3 at one year of age and IgG2 and IgG4 at 2 to 5 years of age. B-cell function remains distinct from that observed in adults throughout childhood (134). By 2 months of age, infants respond well to protein antigens; however, response to polysaccharide antigens does not develop until approximately 2 years of age. The size and number of lymph node germinal centers is also decreased and in mice maturation of follicular dendritic cells is delayed (139). The ontogeny of B-cell responses is influenced not only by limited T-cell stimulation but increasing evidence suggests that the intestinal microbiota plays an important role (140) (see below). Interestingly, the administration of Ig to infants (even premature infants) does not decrease their risk of infection, as has been observed with patients who have Ig deficiencies. This observation underscores the complexity of immune development and the interplay between factors that

renders infants susceptible to severe infections. Beyond Ig, infant complement levels, opsonophagocytic activity, and granulocyte reserves are substantially lower when compared to older children and adults (141).

Despite an innate bias against Th1 responses, when presented with certain antigens, infants can respond robustly and appropriately. For example, bacillus Calmette-Guerin (BCG) immunization elicits a Th1-type response in newborns of similar magnitude to that achieved later in life (142). Similarly, *in utero* cytomegalovirus (CMV) or *Trypanosoma cruzi* infection is associated with mature functional $CD8^+$ T-cell responses (143, 144). Antibody-specific B lymphocytes can be primed *in utero*; however, antibody responses to protein antigen, and particularly polysaccharide antigens, are weaker in infants than in adults (134). In sum, the constellation of neonatal responses primarily protects against extracellular bacterial pathogens (145, 146) rather than intracellular bacteria or viruses. Eventually, over early childhood there is a gradual increase in responses that combat intracellular pathogens and viruses (146). However, under certain conditions infant can overcome their 'bias'. The biologic basis of why infants develop 'mature' responses to some antigens and not other (timing of exposure, route, dose, and type of antigen, activation of APC, etc.) remain elusive.

Gastrointestinal development

The architecture of the intestinal mucosa is established early in human fetal life, and the gut-associated lymphoid tissue (GALT) generally develops in parallel with other lymphoid organs (147) (Fig. 8). However, it is only in the last few weeks of gestation that lymphoid components begin to assume the organizational structures that facilitate appropriate interactions and cross-talk. The development of germinal centers in the Peyer's patches, IgA plasma cells in the *lamina propria*, and the dominance of $CD8^+$ intraepithelial cells occurs late in the third trimester and continues in the post-natal period (147). Studies of CD8-knockout mice suggest these cells are crucial for the downregulation of enterically elicited mucosal immunity (148). Marked changes in the expression of innate immune response genes on enterocytes also occur late in gestation. Studies of fetal, premature, and term infant intestines have revealed developmental downregulation of TLR cell surface expression and associated signaling molecules and upregulation of negative regulators shortly before birth (149, 150). These developmentally regulated changes appear to contribute to the propensity of the immature intestine to respond to stimuli with an excessive inflammatory response (149). Clinically, this response is manifested as necrotizing enterocolitis (NEC). NEC is a severe form of enteritis observed almost exclusively in premature infants and very rarely in term infants. Although the pathogenesis of this disease is complex, the interplay between an immature exaggerated response to stimuli and the process of bacterial colonization is believed to be central to its development (151).

At birth, the infant intestine begins its two critical functions: nutrient assimilation and maintaining immune homeostasis while being challenged with an extraordinary number of antigens. To deal with the antigenic onslaught, two general mechanisms have evolved. The first prevents these antigens from penetrating the mucosal barrier and is accomplished by physical means (e.g. tight junctions, mucin, epithelial receptors), secretory antibodies (IgA and IgM), and various non-specific innate factors. The second mechanism involves limiting the immune response to innocuous environmental and dietary antigens by means of suppressing proinflammatory responses. The newborn must meet these challenges with a permissive mucosal barrier and a naive immune system that must learn to distinguish 'friend from foe'. Thus, immune homeostasis is the balance between epithelial 'leakiness' and suppressing immune responses. The major stimuli influencing postnatal development of mucosal immune homeostasis appear to be the establishment of intestinal microbiota and the timing and dose of dietary antigens (152).

The infant gut is immediately seeded with bacteria, and these initial bacterial encounters appear to have long-lasting immunologic and metabolic effects (153–155). For example, infants born via cesarean section harbor distinct bacteria from those delivered vaginally (156) and have delayed intestinal colonization by *Lactobacilli*, *Bifidobacteria*, *Bacteroides*, and other bacteria (157, 158). These differences may account for the increased incidence of asthma and inflammatory bowel disease in children born by cesarean section (159). Microbial colonization is a complex and dynamic process, and an adult microbial community structure is not established until around 2 to 3 years of age (160). Major factors influencing the infant gut microbiota appear to be genetics, mode of delivery, diet, and exposure to antibiotics (161, 162). The seeding of the infant intestine is facilitated by low gastric acid and pancreatic enzyme production, which allows pathogens including HIV access to the intestinal mucosa (26). The overlying mucin and epithelial receptors on neonatal enterocytes have a distinct glycosylation pattern that changes over time especially during weaning and are believed to be important for guiding microbial succession.

Role of microbiota in intestinal development

Bacteria play a central role in the establishment and regulation of epithelial barrier function as well as GALT maturation in both mice and humans. Over the first few months of life, rudimentary infant Peyer's patches become seeded with IgM and IgA and plasma cells and germinal centers develop. Adult numbers of Peyer's patches are not attained until adolescence (163). Early infant colonization with *Bacteroides fragilis* has been associated with maturation of humoral responses (164, 165), and in murine models, this organism promotes Treg cell development and decreases inflammation (166). By 6 months of life, the infant gut has undergone marked structural and biochemical changes as well as major shifts in the microbiota, enabling it to digest foods other than breast milk or formula.

The role of breastfeeding in shaping the composition of infant stool has been appreciated since the early 20th Century and today is being refined with culture-independent techniques. Not only does the stool bacterial composition differ between breast-fed and formula-fed infants, the expression of immunity-related genes in intestinal epithelial cells also differs (167). The factors in milk that contribute to these differences are an active area of investigation but likely will be numerous and multifaceted. Unlike formula, breast milk is not sterile. It contains hundreds of bacteria (e.g. *Lactobacilli*, *Bifidobacteria*) that appear to traffic to the mammary gland from the maternal intestine (168, 169). Breast milk also contains thousands of bioactive molecules including immunocompetent cells, antibodies, lysozyme, lactoferrin, fatty acids, antimicrobial peptides and glycopeptides, which can inactivate pathogens individually, additively, or synergistically (170). Bacterial colonization is also shaped by the presence of hundreds of unique human milk oligosaccharides (HMOs). HMOs are the third largest constituent of milk and require approximately 10% of the maternal caloric intake for milk production. Such a large expenditure of energy may seem to be an evolutionary folly since the infant cannot digest HMOs. However, it is now appreciated that this complex mixture nourishes the commensal bacteria prevalent in the feces of breastfed infants (171). In addition, HMOs modulate intestinal epithelial receptors, microbe attachment, and cellular responses to stimuli (172).

The stools of breastfed infants are enriched with bacteria that decrease intestinal inflammatory responses, including innate immune mediated inflammation and influence the balance between Treg and Th17 cells (173). These bacteria, along with other factors in breast milk, promote intestinal homeostasis and decrease inflammation. The use of breast milk has transformed the treatment of NEC, and studies using probiotic bacteria in milk are being used in clinical trials (174). Breast milk decreases the risk of developing NEC by 58 to 83%, and even partial breastfeeding provides a significant benefit (175). These effects are consistent with studies observing less intestinal inflammation in breastfed children infected

with enteric pathogens compared to those fed formula (176, 177). Breast milk also suppresses the inflammatory response to stimuli when fed to rodents or added to enterocytes *in vitro*, but which factors or combination of factors are responsible is uncertain (178). Nevertheless, the infant microbiota is inextricably linked to breast milk.

Breast milk and the ontogeny of infant immunity

Breast milk contains a cornucopia of factors that promote intestinal epithelial integrity, growth, and maturation. Intestinal permeability is high at birth and decreases progressively during the first week of life. However, intestinal barrier function does not fully mature until age 2 years (179). This relatively leaky gut is believed to promote tolerance by allowing low dose continuous exposure to antigens. Intestinal maturation and epithelial barrier function are greater in breastfed infants, probably due to the presence of trophic factors including epidermal growth factor, insulin-like growth factor, nucleotides, and other substances (180). Despite these leaky guts, infants and young children have no evidence of systemic immune activation. In fact, their cells are remarkably quiescent. In mice, small transient breaches in the intestine produce dominant regulatory T-cell responses that protect the mucosa from inflammation (181). It is possible that the physiologic anatomical leakiness of childhood results in similar responses.

Although breast milk is often viewed simply as ‘food’, lactation first evolved as a protective immune secretion, and only later in our evolutionary history did it assume a nutritional role (182–184). The mammary gland is arguably the most important part of the integrated mucosal immune system, since it ensures the survival of infants and represents ‘immunologic integration between mother and child’ (185). Even in countries with access to antibiotics, vaccines, medical care, and clean water, breastfeeding is associated with a 50% reduction in hospitalization for diarrhea and 30% reduction for respiratory disease (186). The protective effects of breastfeeding are dose dependent and greatest when infants receive exclusive breastfeeding for the first 6 months of life and then continue to breastfeed until 2 years of age (187).

Given its evolutionary importance, it is not surprising that breast milk appears to compensate for infant immune deficits (188). For example, at birth newborns lack secretory IgA (sIgA), and IgA-producing plasma cells do not appear for several weeks. Breast milk both accelerates the development of IgA-producing plasma cells and supplies large quantities of sIgA with specificities that reflect maternal microbe exposure (189). Pathogen-specific sIgA directly influences intestinal colonization. Additionally, sIgA inhibits macrophage release of proinflammatory cytokines and plays an important role in intestinal epithelial cross-talk, the balance between Treg and Th17 cells (190). sIgA is known to resist digestion, but the unique physiology of the infant gastrointestinal tract coupled with the buffering ability of breast milk appears to permit delivery of other functional molecules to the infant (191). Breast milk IL-7 concentrations are 100-fold higher than maternal plasma levels, and both thymic size and immune responses to vaccines are reportedly increased in breastfed infants (192). Correlations of breast milk IL-7 concentrations with thymic size and signal-joint T-cell receptor-rearrangement excision circles (sjTRECs) in Gambian children (193) suggest that maternally derived IL-7 traverses the infant gut and is functionally active. Murine studies have demonstrated oral functional IL-7 transfer (194).

Breastfeeding during the introduction of complementary foods is important for the development of oral tolerance (195). Breast milk contains an extraordinary number of factors that modulate both the adaptive and innate immune systems (188) (Table 2). Many of these bioactive molecules (e.g. soluble TGF-β, IL-10, anti-oxidants, anti-proteases, cytokines, and fatty acids) are anti-inflammatory and are believed to play an important role in oral tolerance (196, 197). The high levels of IL-7 and IL-15 in milk may contribute to

more robust responses to vaccines observed in some studies comparing breastfed and formula-fed infants (187). Compelling but incomplete evidence indicates that breastfeeding has long-lasting immune effects, including the prevention of allergic and inflammatory diseases (198–202). Thus, breast milk shapes the development of the infant gut as a mucosal barrier, an immune organ, and a site of mucosal immune homeostasis. Breast milk promotes this development by providing immune protection, immune 'guidance', and nutrition (for both the microbes and the infant) in a complex, integrated, and coordinated way that only Mother Nature can accomplish. Any imbalance of this developmental process is likely to have devastating effects.

Effects of HIV on the developing immune system

In the absence of ART, HIV-infected children die at alarming rates. The timing of infection is critical with striking differences in survival between children infected *in utero*, intrapartum, and by breastfeeding (Fig. 1). Additionally, children born to HIV-infected woman who escape infection (HEU-HIV-exposed uninfected) do not escape harm. Thus, HIV has a profound impact on infected children and intriguingly appears to do harm to children born to HIV-infected women that escape HIV infection. The mechanisms by which HIV impacts the developmental changes in infant immunity and how this in turn impacts the natural history of HIV are poorly understood. Below, we review and discuss available data.

Why do infants infected at 34 weeks gestation, birth (37 weeks gestation), or 3 months of age have such markedly different fates? Early studies focused on the quantity and quality of cellular immune responses to HIV, and not surprisingly, infant immune responses were inadequate. HIV-specific $CD4^+$ T-cell responses are detected only sporadically in perinatally infected infants less than 3 months of age (203) and remain low or undetectable even when ART has been initiated in the first weeks of life (204). HIV-specific $CD8^+$ T-cell responses can be detected at birth in infants infected *in utero* but are functionally ineffective (205, 206). In fact, functional $CD8^+$ T-cell responses are not vigorously detected until 3 years of age and only in subjects who have maintained substantial $CD4^+$ T cells (207). Since HIV-specific $CD4^+$ T-cell responses are critical for the induction and maintenance of both effective $CD8^+$ T-cell responses and B-cell activity, the lack of $CD4^+$ T-cell responses may be one explanation for these findings. Active production of HIV-specific ADCC antibodies is observed in the majority of HIV-infected infants only after 12 months of age (208). A decreased capacity of neonatal natural killer cells to mediate ADCC (209, 210) may also contribute to the lack of viremic control. Ineffective immune responses coupled with the large number of $CD4^+$ T cells, furnished by an active thymus, undoubtedly contribute to the high viral loads and the rapid disease progression observed in children. However, these factors are insufficient to fully explain the profound survival differences observed over only a few weeks time.

Until recently, there was limited information on the distribution of HIV target cells in infants. The overwhelming majority of transmitted viruses use the CCR5 co-receptor and productive HIV infection requires activated $CD4^+$ T cells. The majority of infant cells are quiescent, and infants have a remarkable absence of $CD4^+CCR5^+$ T cells in cord blood and only a few in lymph nodes (15, 211). Furthermore, levels of $CD4^+CCR5^+$ expression on peripheral T cells do not achieve adult levels until age 5 years, even in children living in resource-poor settings (211, 212). So, the question remained as to where the target cells of infant infection were located. A recent study of fetal and neonatal intestines found an abundance of memory $CD4^+CCR5^+$ T cells with predominantly Th1 and Th17 phenotypes localized in gut epithelial cells and lymphoid aggregates of the gut submucosa (15). These memory $CD4^+CCR5^+$ T cells were highly susceptible to HIV-infection *in vitro* without prior activation and are likely a prime site of HIV infection, replication, and destruction in infants

and children. Since destruction of $CD4^+$ T cells residing in the gastrointestinal tract is a critical early event in HIV-1 pathogenesis in adults (213–216), and most of the depleted gut $CD4^+$ T cells belong to the Th17 subset (130, 217, 218), it is likely that the gut is an important site of viral replication and destruction in infants as well. There are no human studies of changes in the intestinal tract of HIV-infected infants; however, studies of SIV infection of infant macaques show a rapid, profound, and selective depletion of intestinal $CD4^+$ T cells (219). Rates of $CD4^+$ T-cell turnover and proliferation are much higher in infant compared to adult macaques and may lead to more rapid exhaustion of finite precursor T-cell pools (220). The large $CD4^+$ T-cell pool has been suggested as a mechanism of age-related differences in viral loads and disease progression (220). However, this model most likely oversimplifies the complexity of homeostatic mechanisms deranged by HIV infection. As discussed above, ontogeny of Th17 and Treg subsets in the infant gut are undefined, but the aberrations introduced by HIV infection during this critical developmental window are most likely profound and long-lasting.

In utero versus intrapartum HIV infection

Changes during fetal and neonatal intestinal development may explain the survival differences associated with the timing of HIV infection (median survival 208 days *in utero* acquisition versus 380 days intrapartum acquisition). As discussed above, in response to antigenic stimulation, fetal and preterm intestines have an exaggerated immune response with massive production of IL-8 and other proinflammatory cytokines (221). We hypothesize that the responses of immature enterocytes act as an inflammatory accelerator in the setting of *in utero* HIV infection. The propensity of preterm cells to develop into Th17 cells and the relative paucity of mucosal $CD8^+$ cells in the preterm intestine may also contribute. The net result is high levels of HIV replication and disruption of the regulatory mechanisms governing the epithelial barrier and nascent germinal center development. This disruption likely overwhelms the homeostatic mechanisms that govern Th1/Th2/Th17 intestinal balance, thus leading to even more infections and destruction. This devastation of critical developmental processes leaves an already vulnerable child defenseless.

Although term infants possess the same permissive T cells as the preterm infants, term enterocytes respond to stimuli in a qualitatively and quantitatively different manner. This developmental evolution is believed to be key in the pathogenesis of NEC and may also contribute to the survival advantage of intrapartum versus *in utero* infected infants. Nevertheless, the immunoregulatory networks at birth are fragile and a newborn's innate and adaptive immune responses are ill equipped to control viruses, let alone one like HIV (146). HIV's predilection for the intestine and $CD4^+$ T cells place the infected newborn at a distinct disadvantage. The newborn is immediately assaulted with billions of bacteria and other antigens. During this period, the newborn immune system must 'learn' to shift from a tolerant response to a priming response. In addition, the innate and adaptive arms of the immune system are learning to integrate and cooperate to maintain homeostasis. The gastrointestinal tract is the 'school' where the infant immune system learns many of these lessons and that education is undoubtedly disturbed by HIV infection.

As discussed above, mothers and their milk play a vital role in their children's immunologic development therefore it is probable that maternal HIV infection interferes with their ability to 'educate' their offspring. Understanding how maternal HIV influences infant development is crucial for the design of appropriate interventions. Whether HIV infection perturbs the maternal microbiota and how that might skew infant microbial succession is unknown. Differences in human milk oligosaccharide composition between HIV-infected and uninfected women suggest that there may be differences (73). At a minimum, HIV infection would be expected to impact the reciprocal nature of the regulation between the immune system and microbial community structures. Dysbiosis and alterations in the Th1/

Th2 balance have been associated with the development of allergic disease in HIV-negative children and this may account for the increased rates of asthma and atopy described in perinatally infected children (222, 223).

In adults, HIV infection has been associated with altered barrier function and microbial translocation. Although the role of microbial translocation in driving immune activation is controversial, there are fundamental differences in infant intestinal epithelial barrier function. Notably, intestinal leakiness is the physiologic norm, and this state is probably important for the development of immune tolerance. Presumably this is why infants are hyporesponsive to LPS (117). Although there are few studies in HIV-infected children, available data indicate that the effects of microbial translocation in young children are different from those observed in older individuals. A study of South African formula-fed infants detected circulating LPS in children less than 6 months regardless of HIV infection and LPS levels decreased to undetectable levels in all children at around 1 year of age (224). Although HIV-infected children less than 6 months had higher LPS levels than HIV-negative children, LPS levels did not differ between ARV-naive and ARV-treated children. Elevated LPS levels were also observed in healthy American children less than 2 years of age at levels similar to age matched HIV-infected children (225). However, in children over age 2 years, LPS concentrations were higher in HIV-infected children compared to age-matched healthy controls. In this cohort, levels of LPS and soluble CD14 (sCD14) remained elevated in the HIV-infected children even after optimal viral suppression, CD4 cell recovery and resolution of lymphocyte activation. (225). In both the South African and US HIV-uninfected children, high levels of LPS were not associated with increases in other inflammatory markers. These data suggest marked age-related differences in responses to circulating microbial products.

Postpartum HIV infection

Compared to infants infected *in utero* and intrapartum, the infant infected by breast milk has the advantage of acquiring HIV in the context of a relatively more mature immune system and intestine. After 3 months of age, the ability of infants to successfully fight a variety of infections is markedly improved. By 4 to 6 months of age, the intestine has matured enough to ingest food antigens without eliciting an inflammatory response. Furthermore, as discussed above breast milk contains antimicrobial, anti-inflammatory and immunomodulatory agents that decrease the incidence of infection and accelerate somatic growth and immune development. Systemically and especially within the intestine, breast milk is associated with an anti-inflammatory environment, which would be predicted to attenuate HIV replication and its collateral damage. For these reasons, it is not surprising that rates of disease progression and death are much lower in breast milk-acquired HIV infection.

In Africa, some experts counseled mothers to stop breastfeeding their HIV-infected infants due to concerns of HIV super-infection and possible disease acceleration. However, two studies evaluating survival in breastfed HIV-infected infants show remarkably similar survival curves with a clear survival benefit in breastfed HIV-infected infants (40, 226) (Fig. 2). An Italian retrospective study in 1990 reported that the median progression to AIDS was 9.7 months for formula-fed children and 19.0 months for breastfed children (p=0.003) (226). Survival from diagnosis was also significantly longer in the breastfed infants. The Zambia Exclusive Breastfeeding Study (ZEBS) was conducted over a decade later, but in both studies ART was not widely available to the study subjects. In ZEBS, women were randomized to a group instructed to stop breastfeeding at 4 months or a group instructed to continue breastfeeding for a duration of their own choice (40). All children were given co-trimoxazole prophylaxis, and those randomized to breastfeeding cessation were provided with formula and a nutritionally replete weaning cereal. There was no significant mortality

difference in children with confirmed HIV infection prior to randomization. However, among HIV-infected children who were alive at 4 months, mortality rates by 24 months were 73.6% for children randomized to weaning compared to 54.8% in the control group who continued to breastfeed (P=0.007). Median survival was only 8 months in those randomized to breastfeeding cessation compared to 17 months (p=0.02) in those randomized to continue breastfeeding (40).

Although the survival benefit associated with breastfeeding for HIV-infected infants is extraordinary, these results echo the effects observed in HIV-negative populations (187). A protective benefit of breastfeeding has also been observed in HEU children (227–231). In ZEBS, HEU children randomized to stop breastfeeding had higher rates of morbidity and mortality compared to those randomized to continued breastfeeding (40). Multiple randomized trials (40, 232–235), historical controls (236–238), and epidemiologic studies (239–247) have shown increased morbidity and mortality of uninfected infants born to HIV-infected mothers when breastfeeding is curtailed, as recently reviewed elsewhere (248). Discontinuation of breastfeeding has adverse effects even when combined with safe water interventions (237). The causes of death among the HEU are those that claim the lives of children globally—sepsis, pneumonia, and diarrhea. Presumably these are the pathogens that have plagued humans throughout history, so breast milk has evolved to provide protection. Defining the ‘missing’ components may provide clues as to how HIV disturbs mucosal immunity and the elements of maternal immunity that protect children from infection.

The protective effects of breastfeeding are less in HEU infants compared to infants whose mothers are HIV negative (249). Among HEU infants, the benefits of breastfeeding have been consistently associated with the severity of maternal disease, including in a large meta-analysis (250–253). HEU children whose mothers have lower $CD4^+$ T-cell counts still derive significant benefits, although the benefits are of a smaller magnitude compared to women with higher $CD4^+$ T-cell counts (234). Given the myriad of immunologic factors in breast milk, it is not surprising that the milk of severely immunodeficient women is not immunologically replete. To date, there have been few studies evaluating the immune ‘competence’ of HIV-infected women and its relation to child health. One study observed little to no differences in breast milk Ig concentrations (total and pathogen-specific) and innate immune factors [secretory leukocyte protease inhibitor (SLPI), lactoferrin and lysozyme], between HIV-positive and -negative women (254). Breast milk levels of CCL28 [also known as mucosae-associated epithelial chemokine (MEC)] were positively correlated with child survival in a small study (255).

Defining deficiencies in the breast milk of HIV-infected women could provide clues not only into how HIV deranges mucosal immunity but also reveal key immune factors that mediate protection against infectious diseases. Breastfeeding is considered the pillar of child health, but the complexity of mechanisms by which breast milk promotes child survival hampers our understanding. The immunologic derangements caused by maternal HIV infection may reveal the biologic basis of this protection.

Uninfected but not unaffected: HIV exposed uninfected infants

With the success of programs to prevent MTCT, there is a growing population of HEU children. Despite their escape from HIV-infection, there is an increasing body of evidence that these children suffer immunologic harm both with and without exposure to ARVs (256, 257) and independent of the effects of breast milk. In resource-poor countries, HEU children have significantly increased morbidity and mortality, and in resource-rich countries these children appear to have immunologic derangements (258–272). It should be noted that maternal infections with pathogens other than HIV have been associated with immune

perturbations in the offspring, even in the absence of transmission (256). Compared to unexposed infants, HEU have lower $CD4^+$ T-cell counts with associated reduced thymic output, decreased efficiency of cloning progenitors, and increased serum IL-7 (266, 270, 273). In response to polyclonal stimulation *in vitro*, production of IL-10 is increased and IL-2 is decreased in HEU suggesting an alteration of T-cell activation (268, 274). Furthermore, decreased production of IL-12 in cord blood cells suggest that the function of APC may also be altered (259). Finally, studies on responses to vaccination are inconsistent (275–279). These differences may in large part be due to differences in maternal health. Deficiencies among the HEU appear to be highly correlated with maternal $CD4^+$ T-cell count, so studies with healthier mothers would most likely report little or no effect while those with women with more advanced disease would find associations. In addition, maternal health appears to influence maternal antibody transfer, which may also influence infant response to vaccination (275, 280, 281).

Although data on immune responses to vaccines are unclear, the clinical effects are not. HEU children born to women with advanced HIV disease are up to twice as likely to die in the first two years of life (97, 251), and this outcome is independent of both maternal morbidity and mortality and the separation of mother and infant due to maternal hospitalizations (282). Additionally, HEU children have more episodes of acute and persistent diarrhea, are 6 times more likely to fail antibiotic therapy when hospitalized with pneumonia than equally sick unexposed infants, have more infections with common neonatal pathogens (cytomegalovirus, group A streptococcus, and group B streptococcus), and have an unexpected number of severe infections caused by uncommon pathogens (*Pneumocystis jirovecii*) (252, 260, 263, 283–290).

A key question in the field is whether maternal ART will improve the health of HEU children. The only study that has made this claim failed to separate out the effects of reduced HIV transmission (291). Although HIV treatment obviously has substantial health benefits, ART does not reverse all the immunologic derangements associated with HIV infection particularly in persons with advanced disease. Non-mucosal T-cell levels and function are restored, but abnormalities in B cells and other innate pathways persist even when $CD4^+$ T-cell counts have returned to normal (292–295). Even in developed countries, HIV-infected adults with low $CD4^+$ T-cell nadirs are at increased risk for pneumococcal disease, despite $CD4^+$ T-cell recovery, and in the developing world, ART-treated adults remain at increased risk for TB (296, 297). Both pneumococcal disease and TB are significant causes of infant morbidity and mortality in areas of the world where HIV is endemic. Maternal immunity particularly through breastfeeding protects HIV-negative children from these diseases. (298–300). Additionally, many markers of immune activation/inflammation do not return to normal levels even after years of viral suppression (301), and some appear to be unaffected by ART (225, 301). Chronic maternal inflammation has been associated with untoward effects on the developing fetus and may also contribute to the abnormalites in HEU infants (256).

Questions about mechanisms underlying the vulnerabilities of HEU infants are not academic; these children experience unacceptable levels of morbidity and mortality and interventions to ensure their health are urgently needed. Given the fundamental link between maternal and infant health, it is likely that the immune dysregulation that persists on suppressive ART will profoundly influence this process. Thoughtful clinical studies should be done to establish whether these vulnerabilities persist despite maternal ART, and biologic specimens from these children should be used to probe pathogenic mechanisms. These studies have potential significance not only for public health interventions but may increase our understanding of child immune development and HIV derangements of mucosal immunity.

Lessons from pathogenic and non-pathogenic primate models of infection

Studies of human infants, particular at mucosal sites, are inherently difficult to perform, and therefore much of our understanding of early pathogenesis comes from primate models of infection. Many important similarities between pathogenic models of SIV and HIV exist and strongly support the use of these models in MTCT: (i) pathogenic SIV and simian-human immunodeficiency virus (SHIV) can be transmitted to infant macaques at different stages of gestation including *in utero* and during breastfeeding (302–311), (ii) infant macaques progress more rapidly (usually within 6 months) to simian AIDS (SAIDS) than adult macaques, and (iii) *in utero* transmission is associated with more severe disease than breast milk transmission (312). Furthermore, like human fetuses, infant macaques have a higher prevalence of Tregs in the peripheral blood and lymphoid tissues, which might interfere with SIV-specific $CD4^+$ T-cell responses, subsequently impairing $CD8^+$ T-cell and antibody responses, therefore contributing to the accelerated disease progression (313). Interestingly, $CD4^+$ T-cell-depleted adult rhesus macaques (RMs) challenged with SIV lack the typical post-peak decline in viremia despite $CD8^+$ T-cell and B-cell-mediated SIV-specific immune responses with a plasma viral load curve similar to HIV-infected infants (314). Therefore, the functional defect in HIV-infected infant $CD4^+$ T cells leads to a viremia similar to $CD4^+$ T-cell-depleted adult RMs. Following both intravenous and oral SIV transmission, infant macaques suffer rapid $CD4^+$ T-cell loss in the intestinal mucosa (315), and the tonsil, esophageal, and intestinal mucosa have been identified as primary replication sites (303, 316, 317). A difference in the localization of memory $CD4^+CCR5^+$ T cells appears to exist between neonatal macaques and neonatal humans with subepithelial localization of $CD4^+CCR5^+$ T cells in the *lamina propria* of neonatal macaques (220) versus epithelial localization of $CD4^+CCR5^+$ T cells in human fetuses and neonates (15). How critical this difference will be in early HIV pathogenesis is unknown. Interestingly, transient infection or occult systemic low-level infection has been documented in both adult and infant macaque models of SIV and SHIV (312, 318–320). Notably, in a few cases of low-level infection, rebound viremia occurred following a year or more of aviremic survival (318–320). The implications of these findings in reference to the recent ‘functional cure’ of an infant (321) still need to be determined (see section on the possibility of cure below). Further study of pathogenic macaque models will increase our understanding of the initial stages of HIV infection in human infants and factors critical to viral control while study of nonpathogenic primate models may elucidate factors necessary to attenuate HIV infection.

Thousands of years of co-evolution with species-specific strains of SIV have led to mostly nonpathogenic infections associated with high levels of virus replication (322–326) in over 40 species of African nonhuman primates (327, 328). Interestingly, MTCT of SIV infection in these ‘natural hosts’ [sooty mangabeys (SMs), African green monkeys (AGMs), and mandrills] is rare, occurring at a rate of <7% in SMs and even less in AGMs and mandrills (329–331). Preliminary evidence attributes the low rate of MTCT to low-levels of target cells in the infants, such as activated $CD4^+CCR5^+$ T cells (329). When natural host infants do acquire SIV infection, levels of viral replication are significantly lower than their adult counterparts in direct opposition to human HIV infection. Whether the decreased viremia is due to decreased levels of immune activation and the tolerogenic fetal milieu or other mechanisms requires further study.

Pediatric response to ART and the possibility of ‘cure’

Effective ART has transformed pediatric HIV into a chronic disease. Early virologic suppression is associated with normalization of B and T-cell numbers and function (204, 332–340). Increased thymic function in HIV-infected children and adolescents enhances their immunologic recovery (341, 342).

Not unexpectedly, the benefits of early ART are most pronounced in younger children who are at greatest risk for death and disease progression (343–348). A randomized clinical trial of perinatally HIV-infected infants (median age 1.7 years), without evidence of immunosuppression (% CD4 cells > 25%, absolute CD4 >1,500 cells/μl), found that early ART reduced mortality and disease progression by approximately 75% (332). In contrast, a randomized clinical trial of early versus deferred therapy in older children (median age 6.4 years) did not detect differences in AIDS-free survival, morbidity, or neurodevelopmental outcome (349, 350). Unfortunately, the low event rate underpowered this study, precluding the detection of significant differences between groups. Moreover, there were too few children under 3 years of age for a subgroup analysis. Although this study is inconclusive, it emphasizes the marked age-related differences in survival and how survivor bias can impact pediatric studies (Figs 4 and 5).

Early infant treatment not only modifies clinical disease progression, it may also influence the size and half-life of latent-viral reservoirs established early in infection. These reservoirs represent a major barrier to eradication (351–357), and early studies measuring the recovery of replication-competent HIV from quiescent $CD4^+$ T cells did not find significant differences between children and adults (358, 359). However, a recent study of early treated children suggests that that early therapy decreases the size and half-life of the resting $CD4^+$ T-cell latent reservoir (360). Perinatally infected adolescents who started ART in the first few months of life have been reported to have lower levels of proviral HIV DNA, replication-competent virus, and residual viremia compared to age-matched children treated later in life (361). Interestingly, in contrast to adults, early-treated children lack detectable HIV-specific antibodies and $CD8^+$ T-cell responses, suggesting a fundamental difference in HIV pathogenesis (361). Finally, a recent case report of a 26-month old infant who received ART from 30 hours of age to 18 months and now appears to be ‘functionally’ cured of HIV infection following ART discontinuation is tantalizing (321). Early treatment in macaques with drug resistant virus has been associated with a functional cure in SIV-infected animals after many years of viral suppression (362). Whether this human case will be confirmed and replicated is uncertain. Previous reports of ‘transient’ infant infection did not survive scrutiny (363).

Although speculative, we hypothesize that in infants, rapid, early inhibition of HIV replication turns the pathogenic paradigm on its head. Developmental changes that place an infant at great disadvantage (high levels of $CD4^+$ T cells with rapid rates of cell turn-over, a predisposition to tolerogenic immune responses coupled with a Th17–cell bias after stimulation and a leaky gut) may be advantageous in the setting of effective antiretroviral suppression. The regenerative capacity and tolerogenic milieu that previously fueled viral replication may now accelerate immune restoration, inhibit subsequent rounds of viral replication, and control inflammatory responses during healing. The propensity of neonatal $CD4^+$ T cells to develop into Th17 cells may help restore the intestinal epithelium and even exert antiviral responses (364). Poorly understood neonatal adaptations that impede immune activation in response to a ‘leaky’ gut may also play a role. The alternative hypothesis that antiretrovirals shield the vulnerable immune system allowing it to mature and control viral replication is inconsistent with the absence of detectable immune responses in early treated children.

It now appears that adults, like children, benefit from therapy early in the course of infection with greater recovery of $CD4^+$ T-cell numbers (365), lower virologic set points, and a decreased size of the latent reservoir; however, the rate of decay of the latent reservoir appears to be unaffected (351). These benefits also appear to be independent of HIV-specific T-cell immunity (366). Whether adults with their age-related immune responses will be able to achieve a functional cure is a question of great interest. However, given the difficulty in

identifying adults during acute infection, the full benefit of immediate therapy or potential cure strategies (367) may be better realized in infants where the timing of infection can be more readily established and immediate ART begun.

Concluding remarks

Pediatric HIV infection and the immune response (or lack of response) to HIV pose unique challenges and offer insight into immunologic factors unobtainable by the study of adult populations. The striking age-related changes in disease progression coupled with an increased understanding of normal immune development may shed new light on the immunopathology of HIV disease. Defining factors in breast milk that mitigate HIV transmission and slow disease progression may suggest new strategies for therapeutic and preventative efforts. Finally, the study of HEU children may provide a window into the limits of ART-induced immune reconstitution.

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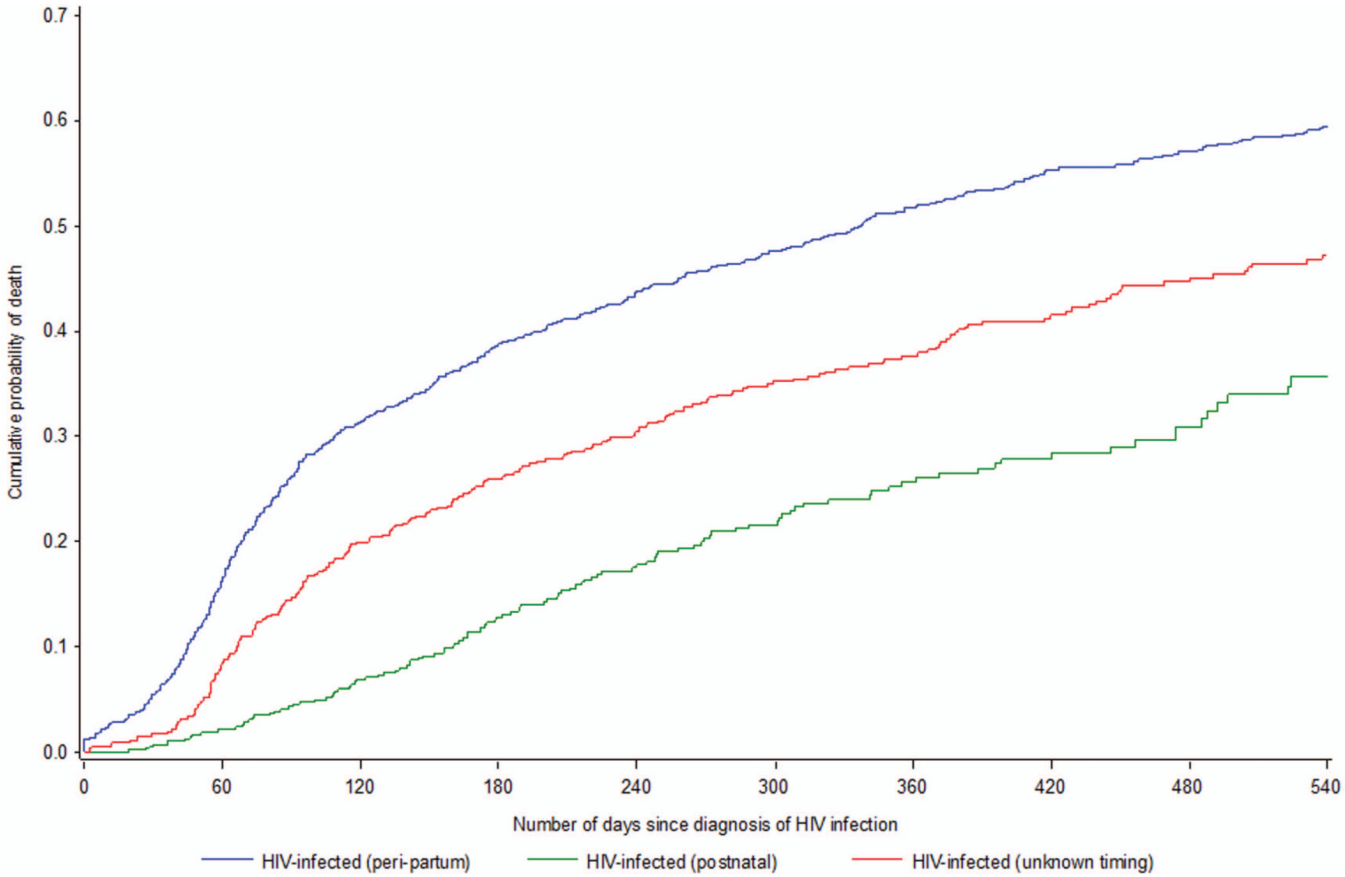

Fig. 1. Estimated 18-month unadjusted mortality for HIV-infected children since acquisition of HIV infection

n = 2,509. from Becquet R *et al.* (99). demonstrating profound mortality differences between peri-partum and postnatal acquisition (52% versus 26% at 1 year).

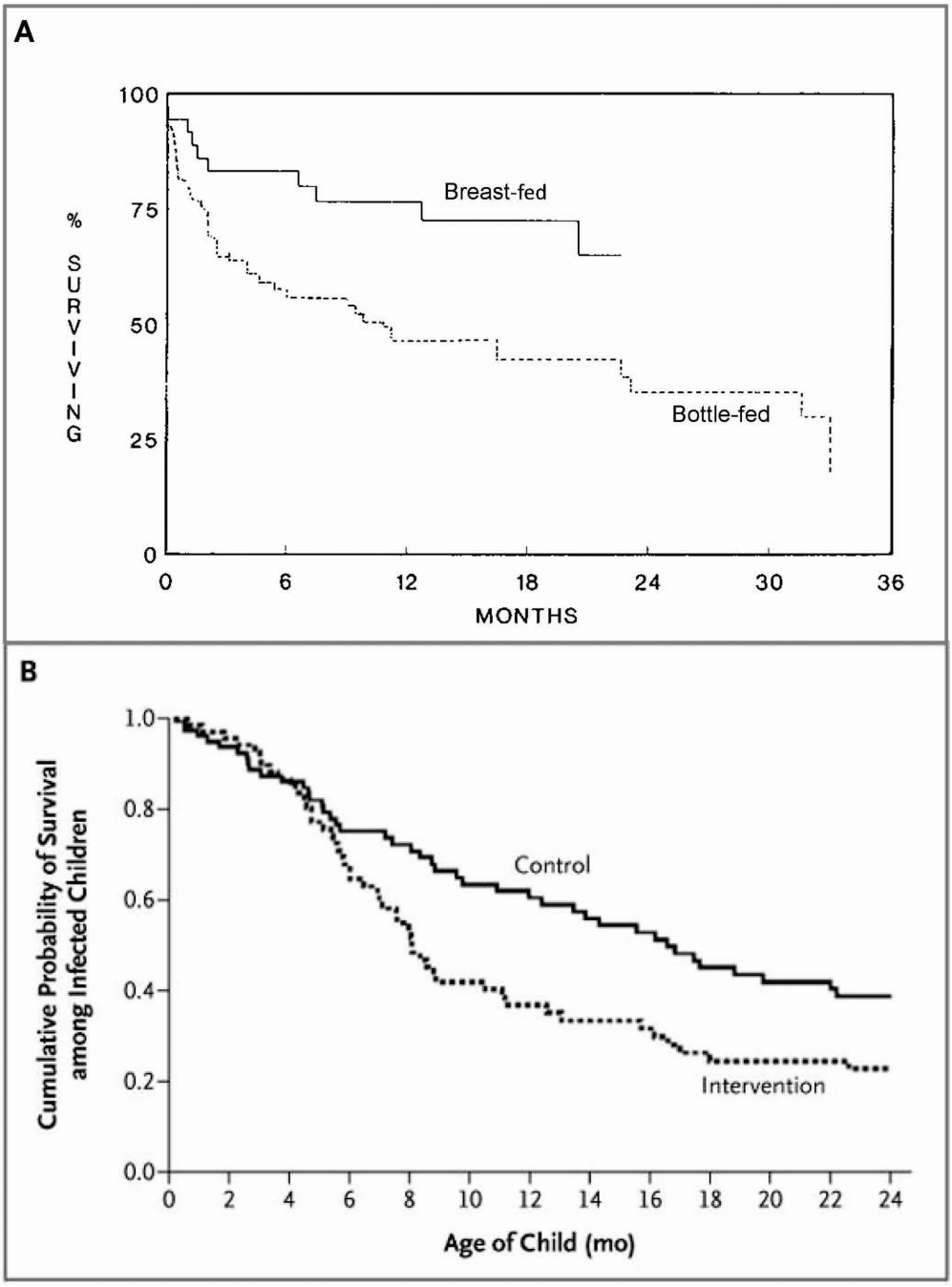

Fig. 2. Survival in breastfed versus bottle-fed HIV-infected infants

(A) Survival to 36 months among HIV-infected infants from diagnosis by type of feeding, 64 bottle fed infants (dotted line) versus 36 breast-fed infants (solid line) from the Italian National Registry of AIDS through February 1990 (Breslow p=0.01; Mantel-Cox p=0.003) from Tozzi *et al.* (240). (B) Survival to 24 months among children who had HIV infection by 4 months of age (71 children assigned to abrupt weaning at 4 months and 81 assigned to the control group of standard breastfeeding practices with median duration of breastfeeding of 16 months) from a controlled trial in Zambia (p = 0.007) from Kuhn et al (40).

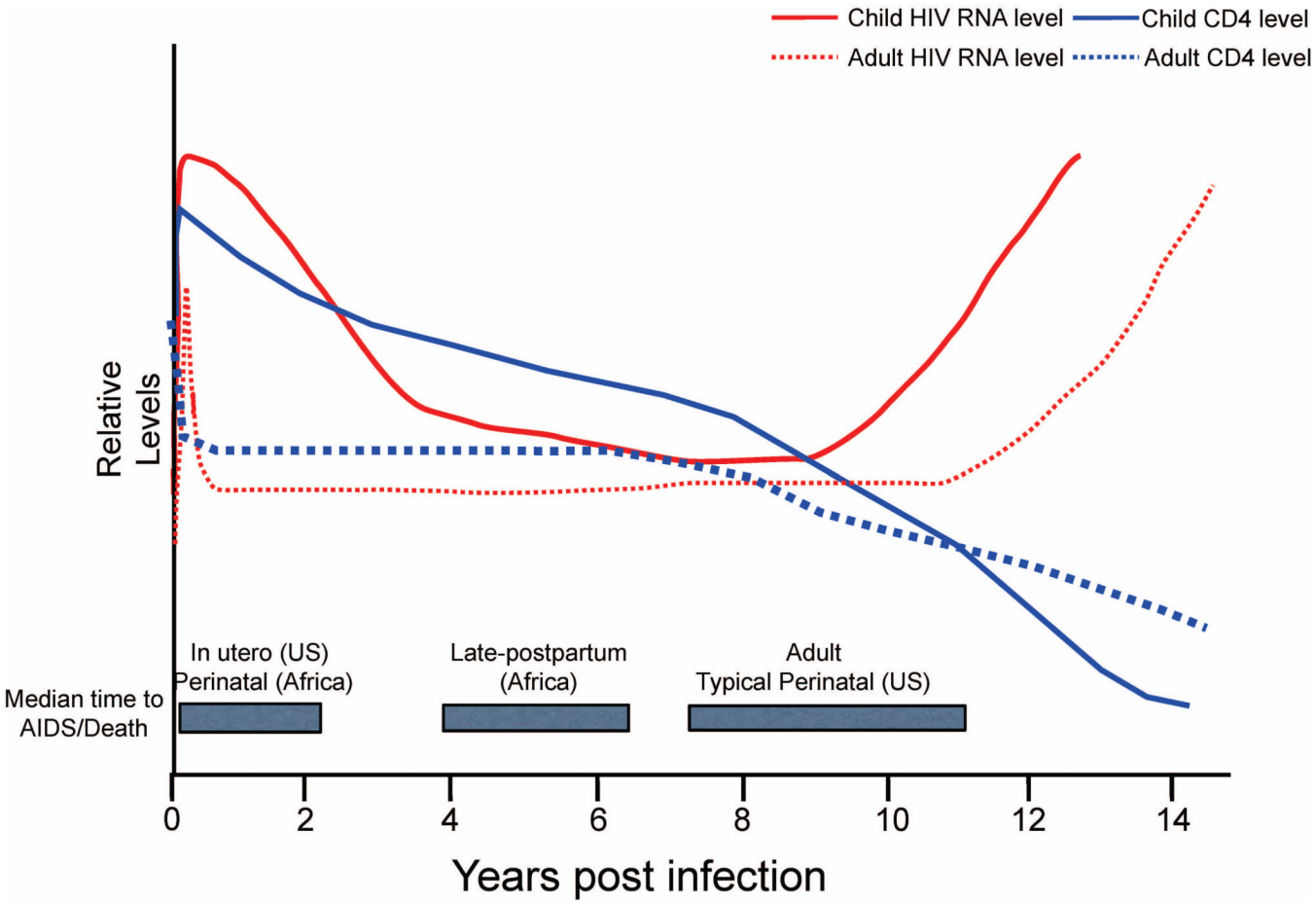

Fig. 3. Relative levels of HIV RNA (red) and CD4 cells (blue) in adults (dotted lines) and children (solid lines) in the years following acquisition of HIV-1

Gray boxes highlight the median time to AIDS/Death for infants by region and route of infection in comparison to adults. US – United States.

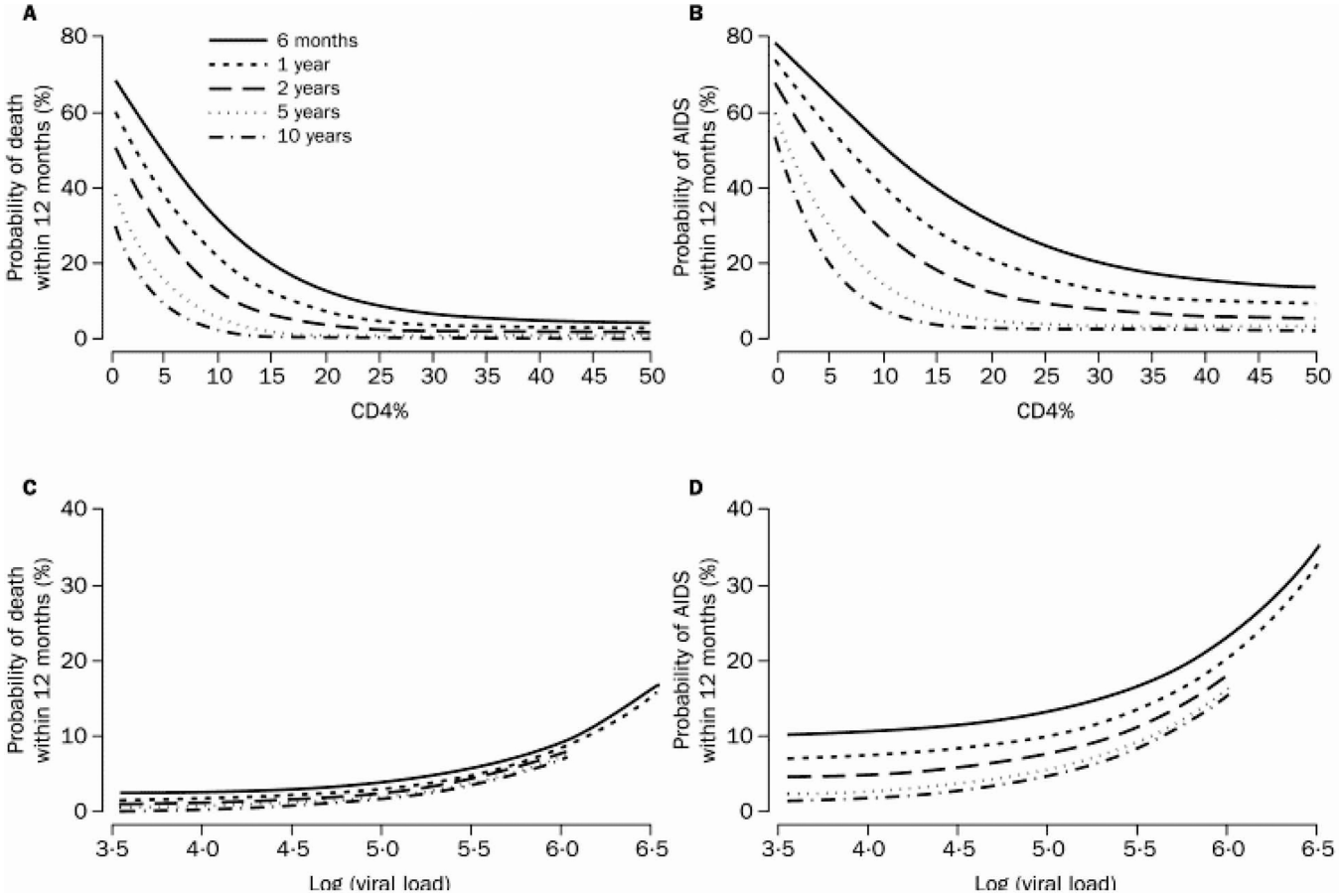

Fig. 4. Distribution of follow-up and events within age groups, for analyses of (A) CD4% and death, (B) CD4% and AIDS, (C) viral load and death, (D) viral load and AIDS by age from the HIV Paediatric Prognostic Markers Collaborative Study Group (101)

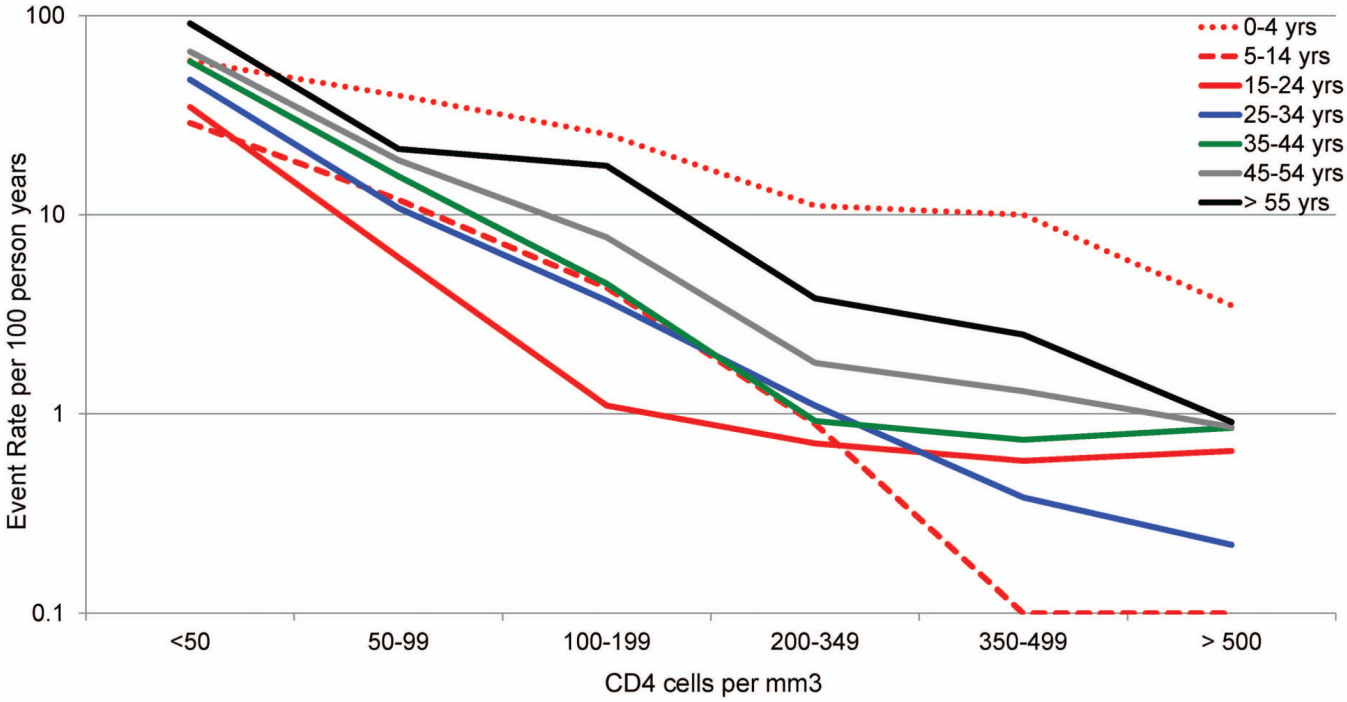

Fig. 5. Death rate per 100 person-years by current CD4$^+$ T-cell count and age

Children aged 0–4 years of age have markedly decreased survival even at high CD4 cell counts whereas adolescent and young adults (15–24 years) have a survival advantage. Adapted from Dunn et al (107).

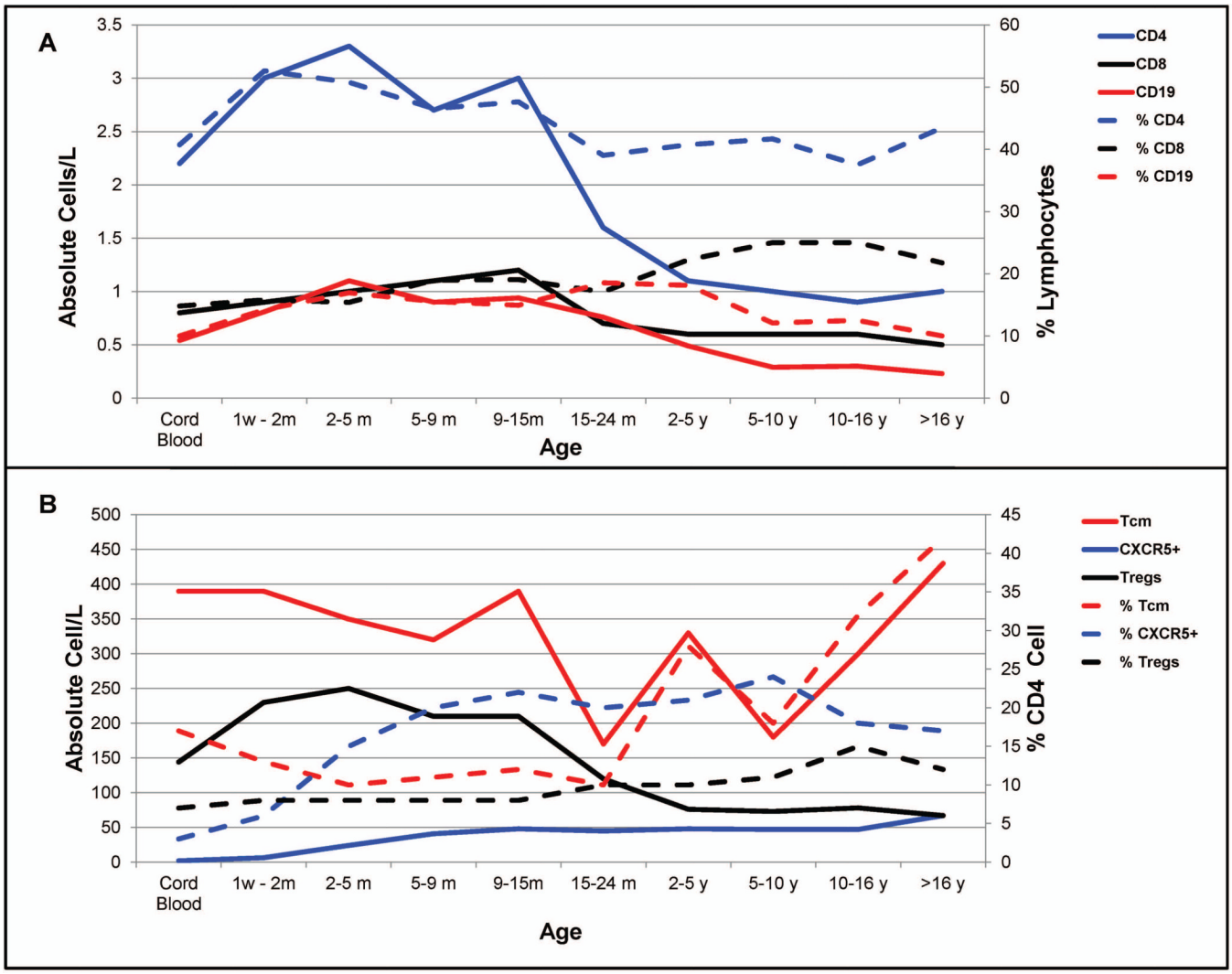

Fig. 6. T-cell populations (Panel A) and CD4$^+$ T-cell population subsets (Panel B) by age

(A) CD4$^+$ T lymphocytes in blue, CD8$^+$ T lymphocytes in black, and CD19$^+$ B lymphocytes in red by number of cells (solid lines) and percentage of lymphocytes (dotted lines) by age. (B) Tcm = central memory T cells (CD3$^+$CD4$^+$CD45RA$^-$CD27$^+$) in red, CXCR5 = CXCR5$^+$ memory helper T cells (CD3$^+$CD4$^+$CD45RO$^+$CD185$^+$) in blue, and Tregs = regulatory T cells (CD3$^+$CD4$^+$CD25$^+$CD127$^-$) in black by number of cells (solid lines) and percentage of CD4$^+$ T cells (dotted lines) by age. Adapted from Schartorje *et al.* (136).

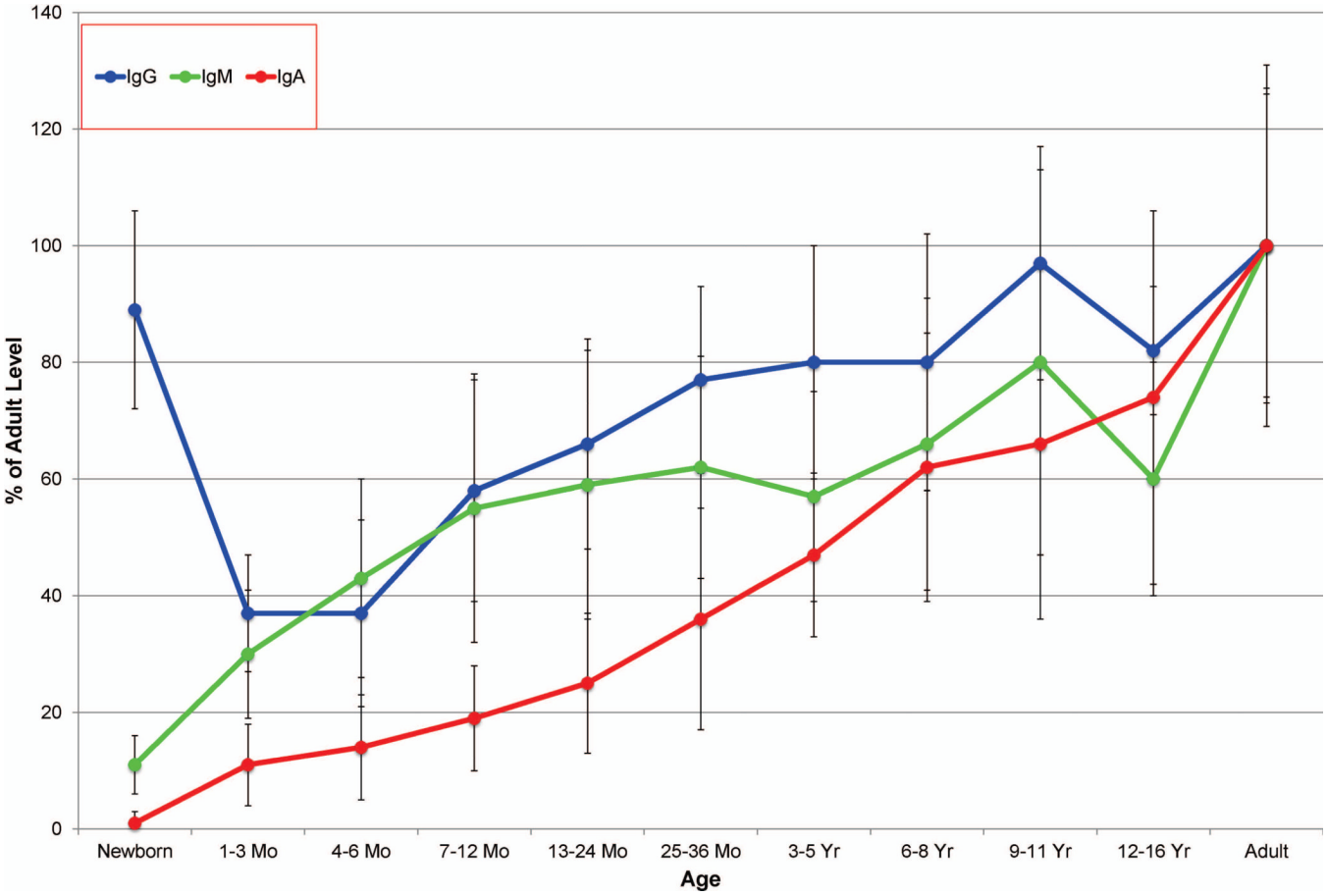

Fig. 7. Percent of adult immunoglobulin levels by age for IgG (blue), IgM (green), and IgA (red) showing significant reduction in immunoglobulin levels in children persisting into adolescence
Adapted from Stiehm (138).

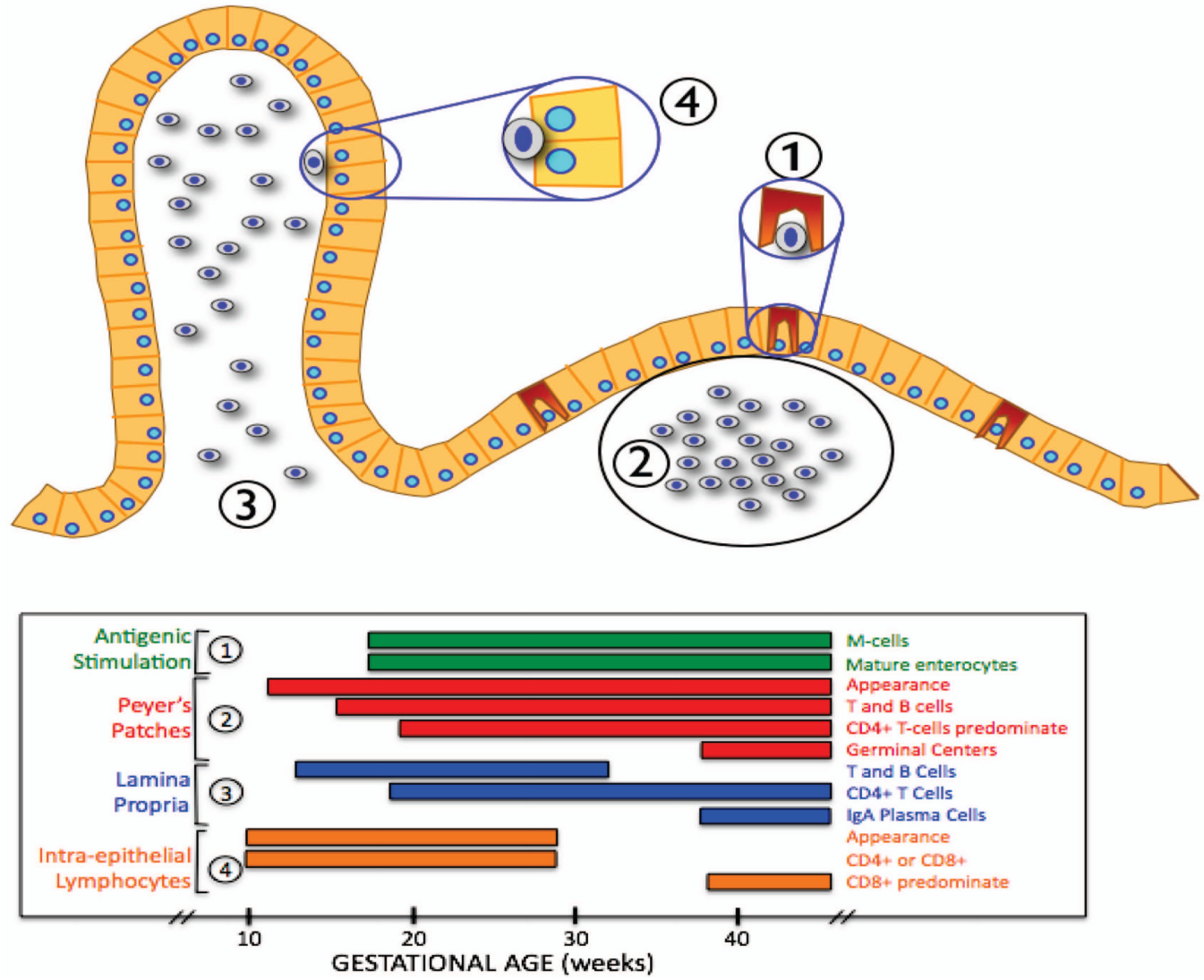

Fig. 8. Development of gut and its immune system with appearance of cellular factors by gestational age

From Insoft *et al.* (147).

Table 1

Risks factors for mother-to-child transmission of HIV-1

Increased transmission	Potential mechanism if known
*In Utero (*60, 98, 368, 369*)*	
Recent Acquisition of HIV-1	Greater exposure to virus Decreased maternal specific HIV-1 immune responses
Maternal Viral Load (DNA/ RNA	Greater exposure to virus
Maternal Hard Drug Use	Greater exposure to virus due to non-adherence ?placental breaches ? increased inflammation
Maternal gonorrhea	
Low Birth Weight	
Prematurity	? Lower levels of maternal immunoglobulin ? exaggerated intestinal cell response to antigen
Low Maternal CD4+ Count	
Placental factors • Il-4, IL-5, IL-6, IL-7, IL9, eotoxin, IL-1Ra, IFN gamma-induced protein 10 (IP-10) • HLA-G	 • Elevated in cases *in utero* MTCT • Upregulated in cases of MTCT
*Intrapartum (*13, 60, 98, 368–371*)*	
Maternal viral load	Greater exposure to virus
Maternal genital HIV DNA Shedding and cell-free RNA	Greater exposure to virus
Prematurity	
Chorioamnionitis	Inflammation resulting in placental breaches greater number of target cells
Prolonged rupture of membranes	Greater exposure to virus inflammation
Placental malaria	
Maternal Genital Ulcers	Breach of epithelial surface
Vaginal candidiasis	Inflammation
Infant scalp trauma	Breach of epithelial surface
*Breastfeeding (*48–51, 58–60, 68, 239–247*)*	
Prematurity	

Increased transmission	**Potential mechanism if known**
Mastitis	Inflammation
Breast abscess	Inflammation
Cracked or bleeding nipples	Breach of epithelial surface
Non-exclusive breast feeding	See text
Parity	
Oral thrush	Inflammation
Other Factors (372, 373)	
Toll-like receptor 9 polymorphisms	Suggests role innate immunity
Maternal-infant HLA concordance	? virus pre-selected
Decreased Transmission (41, 49, 68–70, 73, 374–376)	**Potential Mechanism if known**
Cesarean delivery	Decreased mucosal exposure
Antiretroviral therapy	Decreased exposure to virus, ? other Other unknown
Certain CCR5 polymorphisms (infant)	
Protective HLA subtypes	
Beta-defensin-1 Polymorphisms	Suggests role innate immunity
Placental factors • Placental Hofbauer Cells (macrophages) • Human beta defensins • Leukemia inhibitory factor (LIF)	 • Anti-inflammatory (induce IL-10 and TGF-beta) and restriction of viral transcription • ? inhibits viral replication • ? inhibits viral replication
Decidual cells of the uterine mucosa	Secrete soluble factors
Breast milk factors • Low levels of IL-2 • High Levels of IL-15 • Alpha-defensins • Higher concentrations of non-3' -SL Oligosaccharides • soluble Toll-like receptor 2 (sTLR2)	Immunomodulatory Effects

Table 2

Immune factors in breast milk

Cells	T cells—$CD4^+$ and $CD8^+$
	B cells
	NK cells
	Gamma delta T cells
	Stem Cells
	Macrophages
	Neutrophils
Immunoglobulins	Immunoglobulins sIgA (11S and 7S)
	IgG, IgM, IgE, IgD
	Free secretory component
	Anti-idiotypes
	Anti CCR5 antibodies
Cytokines, chemokines, and receptors	Interleukins (IL)
	IL-1β, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12, IL-13, IL-15, IL-16, IL-18
	Adipokines
	leptin, adiponectin, resistin and ghrelin
	CC Chemokines (Chemokine (C-C motif) Ligand)
	CCL2 (aka monocyte chemotactic protein-1 (MCP-1))
	CCL3 (aka Macrophage inflammatory protein-1α (MIP-1α))
	CCL5 (aka RANTES (regulated on activation, normal T cell expressed and secreted))
	CCL11
	CCL28
	CXCL8 (IL-8)
	CXCL9
	CXCL10 aka Interferon gamma-induced protein 10 (IP-10)
	Anti-proliferative cytokines
	TGFβ1 and -2 (Transforming Growth Factor)
	Proliferative cytokine
	IFNγ (Interferon-gamma)
	G-CSF (Granulocyte colony-stimulating factor)
	M-CSF Macrophage colony-stimulating factor)
	GM-CSF (Granulocyte macrophage (CSF))
	CXCL1 (Chemokine (C-X-C motif) ligand 1)
	eotaxin
	TNF (Tumor necrosis factor, aka TNF-α, cachexin)
	Receptors
	sCD14 (Soluble CD14)
	TLRs (Toll-like receptors),
	sFas (Fas Receptor)

	sFasL (soluble Fas Ligand)
	IL-1 Receptor antagonist
	soluble TNFα receptors I and II (Tumor Necrosis Factor alpha)
	ICAM-1 (Soluble intracellular adhesion molecule 1)
	VCAM-1 (Soluble vascular cell adhesion molecule 1)
Regulators of gene expression Innate Immunity	miRNA
	Exosomes
	Complement C1–C9)
	properdin factors)
	MBL (mannose binding lectin)
Anti-bacterial and Anti-viral factors	β-defensin-1
	Fatty acids
	Haemagglutinin inhibitors
	lactoferrin
	Monoglycerides
	Secretory leukocyte protease inhibitor (antileukocyte protease; SLPI)
Hormones and Growth Factors	epidermal growth factor (EGF),
	nerve growth factor (NGF),
	insulin-like growth factors (IGFs),
	vascular-endothelial growth factor
	α-fetoprotein (glycoprotein)
	Adrenocorticotropin
	Bombesin
	Corticoid-binding protein
	cortisol
	erythropoietin (glycoprotein)
	Glucocorticoids
	Gonadotropins
	Insulin
	Neurotensin
	Ovarian steroids
	Prolactin
	Prostaglandins E1, E2, F2 alpha
	Relaxin
	Somatostatin
	Thyroid-releasing hormone
	Thyroid-stimulating hormone
	Thyroxin
Anti-oxidants	α-tocopherol
	β-carotene
	catalase

	glutathione peroxidase
	lutein,
	vitamin E
Anti-adherence substances	glycolipids (Gb3, Gb)
	glycosaminoglycans
	chondroitin sulfate
	kappa-casein
	Lactadherin (milk fat globule-EGF factor 8)
	Mucins
	MUC1 (Mucin 1, cell surface associated)
	Oligosaccharides
	Fucosylated oligosaccharides
	Sialyllactose
Electrolytes, Vitamins, Minerals, Trace metals and Amino Acids	Biotin
	Boron
	Calcium
	Chloride
	Choline
	Copper
	Fluoride
	Folate
	Glutamine
	Inositol
	Iodine
	Iron
	Maganese
	Magnesium
	Niacin
	Pantothenic acid
	Phosphorus
	Potassium
	Selenium
	Sodium
	Thiamine (B1)
	vitamin A
	vitamin B6
	vitamin B12
	vitamin C
	vitamin D
	vitamin E
	vitamin K
	Zinc

Bacteria (select)	Bifidobacterium bifidum
	Lactic Acid Bacilli
	Streptococcus
	Staphylococcus
	Serratia
	Corynebacteria
Enzymes	antiproteases
	Lactoperoxidase
	leukocyte enzymes
	Lipoprotein lipase
	Lysozyme
	platelet-activating factor-acetyl-hydrolase
	Ribonuclease
	Xanthine oxidase
Carrier proteins	Lactoferrin
	Steroid binding protein
	Transferrin
	vitamin B-12 binding protein
Other (or multiple classifications)	Carbohydrates
	Caseins
	alpha-lactoglobulin
	alpha2-macroglobulin (like)
	(Tri to penta) phosphorylated beta-casein
	Gangliosides (GM1-3, GD1a, GT1b, GQ1b)
	Glycopeptides
	Glycoproteins (other)
	CD59 glycoprotein aka MAC-inhibitory protein (MAC-IP)
	Glycoproteins (mannosylated)
	Glycoproteins (receptor-like)
	Glycoproteins (sialic acid-containing or terminal galactose)
	Heparin
	LALBA (Lactalbumin alpha)
	Lewis Antigens
	Lipids (other)
	Long-chain polyunsaturated fatty acids
	MIP (macrophage migration inhibitory factor)
	Nucleotides
	Phosphatidylethanolamine
	Prostaglandin
	Sulfatides
	Trypsin Inhibitor

Table 3

Neonatal Immune System

Innate Immunity	Increased IL-10 production Decreased IFN-gamma and IL-12 production Increased IL-6, IL-23, and IL-1 beta production Favors extracellular bacterial pathogen protection
Phagocytes	Decreased production of phagocytes Poor adhesion molecule function Abnormal migration across endothelial barriers Inadequate chemotactic response Deficits in hydroxyl radical production Decreased phagocytes at site of infection
Cell-mediated Immunity	Decreased mature memory T cells in blood Restricted development of Th1 lymphocytes Decreased T helper function Increased differentiation of regulatory T cells Increased differentiation of Th2 lymphocytes Decreased cytokine production • IFN-alpha, IL2, IL-4, IL-10 Decreased NK cell ADCC Poor stimulation of B cells
B-lymphocytes and Immunoglobulins	Decreased antibody production Poor isotype switching Decreased Ab affinity Decreased total antibody & antibody subtypes (Figure 7) Deficient opsonization by immunoglobulins Poor response to polysaccharides
Complement Cascade	Decreased function of classic and alternative pathways Low levels of C5a

IL – interleukin; IFN – interferon; Th1 – T helper 1 lympocytes; Th2 – T helper 2 lymphocytes; NK – natural killer cell