Cunningham-Rundles

Impact of Perinatal Nutrition on Neonatal Immune Response

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Shaped by fetal life, the neonatal immune system is immature at birth and must adapt rapidly to new environmental challenges. Newborn exposure to colonizing commensal bacteria, environmental antigens, bioactive dietary substances, and potential pathogens has the potential to cause long-term effects on health.[1,2] Differences in maternal and neonatal nutritional status are increasingly recognized as both a major source of variation in health outcomes and as an avenue for early intervention.

The historical view of neonatal immune response, based on specific studies in mice, was that an early encounter with antigen led to lack of responsiveness, or tolerance to the same antigen, but also conferred susceptibility to infectious organisms and poor response to vaccines. The later discovery of T-lymphocyte subsets and the effects of microbial pattern recognition receptors on innate immune cells has led to a very different understanding of why infants are vulnerable to infection, and also why some infants are susceptible to allergy, asthma, and even obesity and type 1 or 2 diabetes in later life.[3-8]

Current studies show that the newborn immune system has mainly naïve thymus-derived T lymphocytes, lacks memory T and effector B lymphocytes, and has a deficient T-helper (Th) type response characterized by low or absent production of interferon gamma. Although the T-cell response in newborns is now known to vary widely in individual infants, the cytokine response usually is dominated by interleukin-4 (IL-4) and IL-13, a pattern that is characteristic of Th-2 cells. The Th-2 cytokine phenotype forms in fetal life as part of maternal fetal regulation to avoid inflammation and must shift to a Th-1 type at birth. Failure to do so is potentially associated with allergic predisposition.[9,10]

Neonatal host defense depends on innate immune responses to environmental antigens that prime and mold the developing adaptive immune system.[11] However, recent studies show that the innate immune response also must undergo postnatal maturation. For example, expression of a key pattern recognition toll-like receptor 4 (TLR4) on neonatal monocytes is low compared to adults and is a cause of infants' delayed response to lipopolysaccharide (LPS), the dominant surface component on gram-negative bacteria.[12]

The differences between infant and adult immune response are not only greater in premature infants, but also often include a dysregulated cytokine response characterized by increased production of inflammatory cytokines that is not controlled by an equal anti-inflammatory response.[13,14] The preterm infant's susceptibility to unbalanced inflammation is related, at least in part, to reduced progenitors of regulatory T cells (Treg) in the naïve T-cell population (Fig 1).[15]

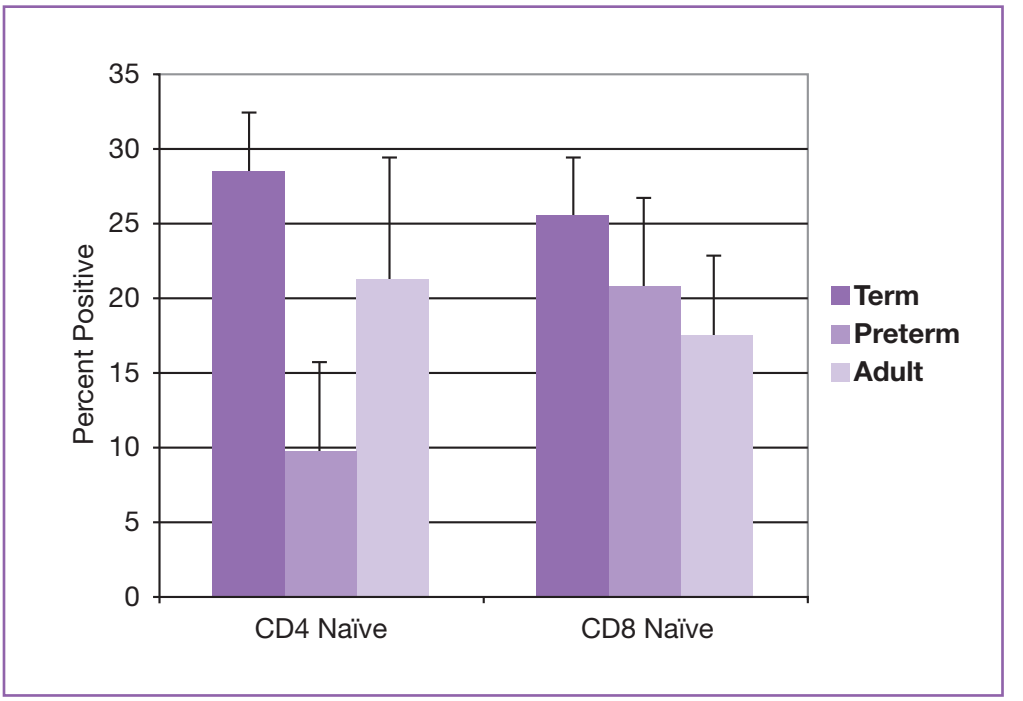

Fig 1. Naïve CD4+ T cells are reduced in preterm compared to term infants. Data show that the population of progenitor cells for T regulatory lymphocytes (CD4+CD25+Foxp3+ and Th-17 T cells) is lower in preterm compared to term infants (P <0.01). The data are shown as percent of cells positive for lineage and memory markers as determined by flow cytometry. This is one possible reason why preterm infants are more vulnerable to infection.[15]

While all of these problems are greatly magnified in the preterm infant who is small for gestational age (SGA), even late preterm infants who are appropriate for gestational age (AGA) and infants <1500 g show greater risk of infection at birth and greater morbidity from infection in early childhood.[16,17] Numerous studies indicate that low birthweight is associated with enhanced levels of proinflammatory mediators, overweight, obesity, and enhanced risk of type 2 diabetes in later life.[2,18] The Barker hypothesis that adult diseases have their origins in early development, such that prenatal undernutrition compromises later function, also is true for antibody formation and thymic function.[19]

Exposure to food (nutrients, antigens, and bioactive substances) and microbes primes immune response and can lead to beneficial adaptive changes, such as production of IgA and IgM that exclude antigens from crossing the gastrointestinal tract, and the timely development of suppressor mechanisms that are needed to mediate oral tolerance to foods and commensal bacteria. For example, cow's milk allergy, because of exposure to casein, sometimes is a serious problem, which causes diarrhea, impairs growth, and often is associated with other atopic conditions. The child's ability to outgrow allergy to cow's milk and become tolerant depends upon the development of casein-specific Treg cells.[20] The causes of allergic predisposition include both genetic and environmental factors.

Atopic Diseases, Allergy, and Asthma

The overall incidence of atopic diseases, allergy, and asthma increased dramatically during the 20th century. This is attributed to decreased early exposure to commensal bacteria and microbes as encapsulated in the hygiene hypothesis.[21] Current studies show that supplementation with probiotic lactic-acid bacteria reduces the development of allergic responses in children with inherited genetic risk and ameliorates response in milk-intolerant children.[22-25] Although more detailed studies are needed, nutrients are recognized as critically important for the development of the microbiota and energy homeostasis.[26,27] The development of future microbiome in the infant occurs during gestation and during early neonatal life, and interacts with genetic and epigenetic factors involved in fetal and neonatal programming.[28]

Protein-Calorie Malnutrition

The impact of primary maternal protein-calorie malnutrition (PCM) on neonatal vulnerability to infectious disease is well known. Much of the damage to neonatal host defense occurs through impact on the developing immune system, especially the thymus, often called the barometer of nutrition.[29] Malnourished children have lower levels of thymulin and deficient T-cell development. Zinc deficiency alone also can cause this.[30] Leptin, the adipocyte-secreted hormone that regulates weight centrally, regulates the thymus by increasing thymopoiesis and inhibiting apoptosis, and is decreased in malnutrition. Malnutrition enhances tumor necrosis factor and IL-1, IL-6, and IL-8 cytokine production, activates hepatic synthesis of acute phase reactant proteins such as C-reactive protein, and inhibits production of serum albumin and transthyretin. Shifts in storage pools of iron, zinc, and copper during the acute phase response because of transport by newly synthesized binding proteins such as ferritin, metallothionein, and ceruloplasmin lead to low levels of

these trace elements in blood. Although malnutrition is associated with reduced cytokine response to antigen in vitro, the levels of circulating proinflammatory cytokines in vivo are increased.[31,32]

Micronutrient imbalance or deficiency in the mother in the absence of PCM can alter the program of immune development in the infant (Table).[33] The strongest evidence for micronutrient programming effects comes from studies of vitamin A deficiency. Vitamin A is required for the homing of T cells into the gastrointestinal tract and promotion of antigen-specific Treg development. Retinol concentrations at birth are associated with atopic disease in childhood and later life.[34]

Table. Micronutrients Modify Neonatal Immune Response[7]

Zinc deficiency:
- Gestation—teratogenic effects
- Levels in milk affect neonatal T cells, natural killer (NK) cells, cytokines
- Persistence of defects after repletion

Iron deficiency:
- Prenatal stress may cause anemia, NK cell defects
- Deficiency increases iron and copper gene expression, decreases oxidative response genes (eg, vitamin C transporter)

Selenium:
- Deficiency may increase viral virulence

Vitamin C:
- Transporter variants cause preterm birth
- Improves response to infection

Vitamin D (deficiency is common):
- Low D_3 promotes infant allergy, atopy, inflammation; repletion (+ Ca) cures experimental inflammatory bowel disease
- Low D_3 increases infections

Vitamin A (deficiency is common):
- Deficiency worsens infection
- Essential for development of the gut-associated lymphoid tissue (GALT)
- Important for oral tolerance

Vitamin E:
- Maternal levels influence allergic sensitivity

Inflammatory Challenge Relates to Gestational Age

Current studies show that the newborn immune system is more vulnerable to inflammatory challenge in relationship to gestational age. Importantly, neonates appear to have a reduced compensatory anti-inflammatory response. Therefore, they are possibly at greater risk for inflammatory damage. Recent studies showed that neonatal cytokine response to bacteria is dysregulated in term and preterm infants compared to adults and has a tendency toward an uncompensated proinflammatory response.[15] Although a lower percentage of neonatal monocytes produced cytokine responses to a panel of microbes compared to adults,[35] the levels of cytokines IL-6 and IL-8 secreted in response to the same microbes actually were higher (Fig 2).[36]

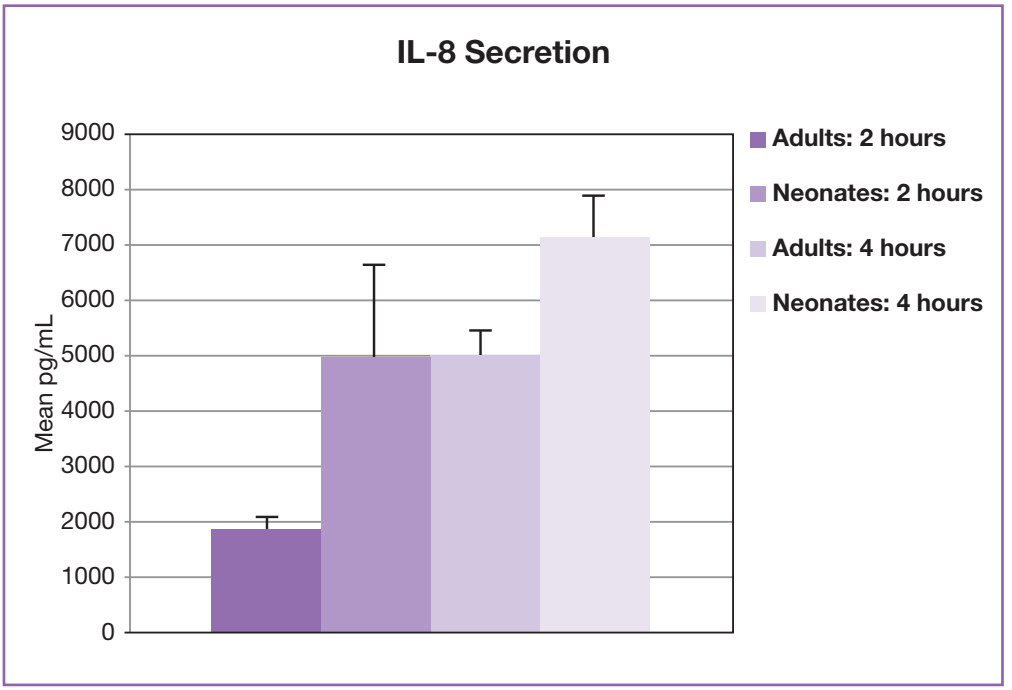

Fig 2. Neonatal cytokine secretion in response to *Escherichia coli.* Data show that neonates secrete more IL-8 at 2 hours in response to physiological heat-killed *E coli* than do adults (P <0.01). Data are shown as picograms (pg)/mL, as measured by enzyme-linked immunosorbent assay (ELISA). Increased levels of cytokines can lead to hyperinflammatory response.[35]

The Potential Role of Polyunsaturated Fatty Acids

Nutrient treatment may offer a physiological and safe approach to promote a balanced proinflammatory response and protect against overproduction of cytokines associated with preterm birth. The potential role of long-chain omega-3 polyunsaturated fatty acids (PUFAs) that are not synthesized de novo is of particular interest because of the evidence for anti-inflammatory activity when given perinatally.[37] Formulas given to preterm infants on total parenteral nutrition do not provide a significant source of either eicosapentaenoic (EPA) or docosahexaenoic acid (DHA). Preliminary data from our studies using the THP-1 human monocytic cell line showed that cytokine responses to LPS were significantly reduced compared to controls when cells were pretreated with EPA or DHA. Subsequent studies with full-term healthy neonatal cord blood also showed that PUFA treatment inhibited proinflammatory cytokine response.[38,39]

Summary

In summary, current studies show that postnatal development of the immune system requires priming signals and nutrient/micronutrient resources. Variation in neonatal response provides a sensitive reflection of genetic and epigenetic factors that interact with the evolving microbiome and are predictive of future response to environmental antigens, bioactive dietary substances, commensal bacteria, and potential pathogens. The need for controlled proinflammatory response and a shift from a Th-2- to a Th-1-dominated cytokine pattern after birth are required to engender tolerance, promote host defense, and avoid allergic responses. Birth weight, gestational stage, and nutrient sufficiency affect immune development.

Micronutrients and microbial encounter can protect against allergic predisposition, and nutrients influence the evolving microbiota, as well as immune development. Conversely, nutrient and micronutrient deficiencies at birth and an abnormal microbiota impair immune regulation and can deprogram immune development. Nutrient supplementation and probiotic treatment are valid approaches for postnatal use to regulate and support neonatal immune response and avoid a hyperinflammatory response.

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Q & A

Q: Can you elaborate a little on intervention, because the results are fine—the interplay between intestinal permeability and the exposure to antigens. Then you have the question of the timing, of when would you expose and when a neonate eats something. It depends on the immune system and comorbidity (potential pathogens) of the gut, and at various times, you will get different responses. Many problems come up with the interaction of immune cells and then the immune response to gut microbes, and there is now increased permeability. Could you elaborate a bit on this issue?

Dr Cunningham-Rundles: That is of concern. I do not know if you are thinking now about the use of probiotic organisms in early life. You would have some concerns about gut permeability there.

What we would propose doing, at least our next step, is preterm infant feeding TPN, total parenteral nutrition. We would propose using, at least in the beginning, DHA. I did not show you the data, but in our ramp-up studies, we discovered the DHA was more powerful at lower doses, so we proposed adding it as a supplement. The addition of DHA actually is beneficial, so it should not present a problem, and we can probably get that through the institutional review board.

I do not think about it having any problems with respect to gut permeability. It would definitely have a potential impact on immune cells as well.

Q: I was referring to cow's milk rather than to probiotics, because the studies are talking about exposure during the first 2 weeks of life and whether you would prefer, in order to create tolerance, that the gut is still permeable or not.

Dr Cunningham-Rundles: I do not really have an answer to that. Primarily, we still think of doing things once you have a problem. You assume that tolerance will develop.

People have different points of view about whether or not you should wait and let children outgrow cow's milk allergy or if you should intervene immediately and put them on another diet. That is another question.

In terms of when best to intervene with omega-3 fatty acids, I think you could give omega-3s as soon as you were giving any element of food other than mother's milk, which of course does contain some DHA.

Q: We have just completed a randomized control trial of increasing salmon intake during pregnancy, contaminant-free salmon and intervention.

We have looked at the innate immune responses to TLR4 and such, IL-10 production, and we did find quite a substantial effect of maternal supplementation with the provision of salmon on the IL-10 responses in the cord blood samples. I think it is potentially a pathway for us.

Dr Cunningham-Rundles: I might quickly add that omega-3 fatty acids are not anti-inflammatory. They are proinflammatory, but much less, so it is a balanced effect. You also get effects from incorporation into membranes. I am so glad that you have done that study, because that was needed, because it is good to do for the mother as well.