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REVIEW

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A role for metabolism in determining neonatal immune function

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Abstract

Immune responses of neonates differ markedly to those of adults, with skewed cytokine phenotypes, reduced inflammatory properties and drastically diminished memory function. Recent research efforts have started to unravel the role of cellular metabolism in determining immune cell fate and function. For studies in humans, much of the work on metabolic mechanisms underpinning innate and adaptive immune responses by different haematopoietic cell types is in adults. Studies investigating the contribution of metabolic adaptation in the unique setting of early life are just emerging, and much more work is needed to elucidate the contribution of metabolism to neonatal immune responses. Here, we discuss our current understanding of neonatal immune responses, examine some of the latest developments in neonatal immunometabolism and consider the possible role of altered metabolism to the distinctive immune phenotype of the neonate. Understanding the role of metabolism in regulating immune function at this critical stage in life has direct benefit for the child by affording opportunities to maximize immediate and long-term health. Additionally, gaining insight into the diversity of human immune function and naturally evolved immunometabolic strategies that modulate immune function could be harnessed for a wide range of opportunities including new therapeutic approaches.

KEYWORDS
immunometabolism, metabolic adaptation, neonatal immunity, T cells, umbilical cord blood

1 | INTRODUCTION

Birth represents a relatively dramatic transition from an environment with a low microbial burden to one abundant in commensal and potentially pathogenic challenges that the neonatal immune system must be able to respond to. Immune responses in preterm and term neonates differ from those of adults, generally being characterized as diminished, tolerant or Th skewed although we must be mindful that these are highly evolved, stage of life appropriate responses. Efforts to clarify the phenotypes and mechanisms prevalent at this time are ongoing so that we can better understand the significance of differences at this stage of development for long-term health. The need to understand how the neonatal immune response differs to that of adults is driven by the mortality and morbidity associated with infection and sepsis in newborns and infants and the need for protective vaccine responses that can mitigate these.

Recent decades have also seen growing appreciation that neonatal immune phenotypes, shaped predominantly by environmental factors during pregnancy, are linked to the later development of non-communicable immunoinflammatory diseases.[1–3] Finally, while the therapeutic capacity of umbilical cord blood is already being harnessed through transplantation, there is growing appreciation of the wider regenerative capacity of neonatal immune cells, for example in the regeneration of heart tissue.[4] Given this diversity, there is a real need to elucidate the mechanisms that uniquely programme neonatal immune cell phenotype.

The development and maturation of lymphoid progenitors during foetal and early post-natal life are influenced by a multitude of factors including cytokine profiles, transcription factors, expression of surface proteins, prostaglandins produced by the placenta and altered levels of metabolites, such as adenosine, in neonatal plasma.[5–7] Maternal immune status during pregnancy also appears to affect neonatal and infant immune development. For example, infants of mothers who suffer from allergic disease have reduced interleukin (IL)-6 production and p38 mitogen-activated protein kinase (MAPK) phosphorylation compared with age-matched healthy controls.[8] It also has been long recognized that maternal and neonatal nutrition have lasting effects on immune development, contributing to the development of allergic disease, metabolic syndrome and chronic inflammatory conditions.[9] Energy demands in early life are extremely high with little to no energy, especially fatty acids and proteins, reserves. The metabolic and energetic demands required for growth in early life severely limit nutrient availability for highly energetically demanding immune responses.[10] Disease tolerance, wherein the goal is to limit disease immunopathology versus the pathogen per se, is suggested as an evolved method of host defence in early life to limit harm to the host in the face of these reduced energy reserves. Energy demands of neonates and the disease tolerance hypothesis have been reviewed extensively elsewhere.[10–14]

Immunometabolism is a relatively recently emerged field of research that seeks to elucidate the crucial role metabolic pathways have in shaping immune cell fate and function. The activation and function of most immune cells is dependent on metabolic reprogramming to a state of aerobic glycolysis to enable effector function. In this setting, the primary fuel is glucose which is metabolized through the central metabolism pathways of glycolysis to pyruvate (Figure 1). Aerobic glycolysis rapidly generates adenosine triphosphate (ATP) via substrate-level phosphorylation as well as reducing nicotinamide adenine dinucleotide (NAD)+ to NADH. Many cells also convert pyruvate to lactate to recycle NADH, thus maintaining glycolytic flux. Glucose metabolism also supports activation of immune cells by generating anabolic intermediates required for cell growth, division and function.[15] Pyruvate enters the Krebs cycle via conversion to acetyl-CoA. NADH and flavin adenine dinucleotide (FAD)H2 are the major products of the Krebs cycle, which support oxidative phosphorylation (OXPHOS) by transfer of electrons to the electron transport chain. Catabolism in the Krebs cycle also supports cell growth, producing the anabolic building blocks for amino acids and lipids. To support catabolism, the Krebs cycle must be replenished, in a process called anaplerosis, converting various substrates such as the amino acid glutamine into Krebs cycle intermediates (Figure 1). Fatty acid oxidation (FAO) also feeds into the Krebs cycle via acetyl-CoA as well as supporting OXPHOS through NADH and FADH2 (Figure 1).

Key Message

Despite great advances in our understanding of immunometabolism, few advances have been made in applying these insights to human neonatal immune responses. Emerging evidence suggests a central role for metabolism in defining the immune responses of neonates and these could underpin functional differences implicated in the development of atopic disease for example. Here, we summarize the latest findings on immunometabolism and highlight gaps in the literature with specific regard to neonates, exploring connections between metabolism and neonatal immune phenotype. A better understanding of immunometabolism in neonates could have impacts ranging from improved health to new regenerative treatments.

The differential utilization of fuels, including in competition with other cells in the tissue microenvironment, can impact on cell fate and function.[16] We suggest that immunometabolism provides a framework for understanding phenotypic and functional differences in the neonatal and adult immune systems and also between neonates linked to individual disease susceptibility. While data supporting this are emerging, these studies are still very much in their early days and there are many unanswered questions. In this review, we will discuss the current understanding of differences between neonate and adult immune responses; how metabolism may influence these changes and the further work that is required.

2 | INNATE IMMUNE RESPONSES

The innate immune system provides front-line cellular and humoral defence through the release of cytokines and other mediators that amplify the immune response, recruitment of first responders and education of the adaptive immune response to help counter the threat and develop immunological memory. These processes demand energy and biosynthetic intermediates that are generated via cellular metabolic pathways.

2.1 | Neutrophils

Neutrophils comprise 70% of the leukocytes in blood. They extravasate from the blood and quickly travel to the site of insult along a chemokine gradient typically released by tissue-resident macrophages. Once in the tissues, neutrophils engulf and kill pathogens

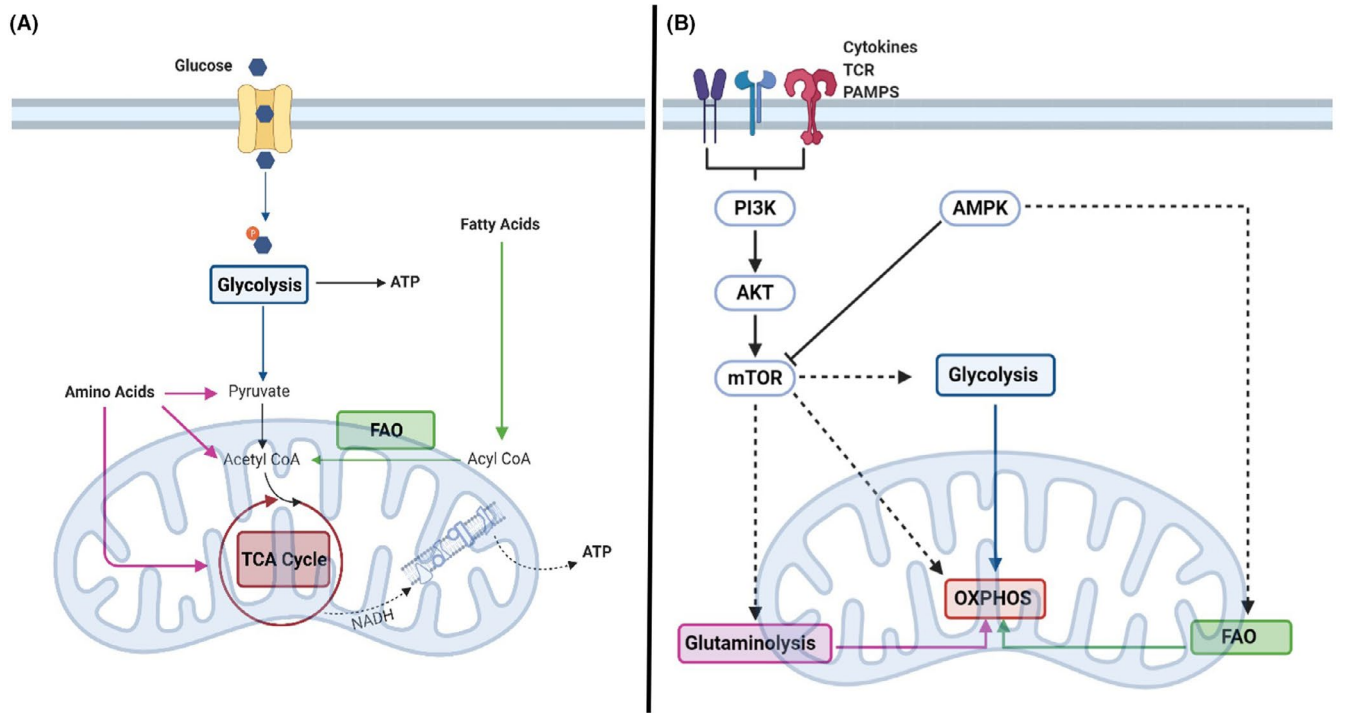

FIGURE 1 Basic metabolism and metabolic signalling pathways. A) Glucose, amino acids and fatty acids are the major metabolites utilized by cells of the immune system. These metabolites feed into the TCA cycle, through glycolysis, fatty acid oxidation or other metabolic pathways. Oxidation of these metabolites, through these metabolic pathways, generates ATP via substrate-level phosphorylation or through the electron transport chain. B) Metabolic pathways are influenced by a number of external stimuli including cytokines, specialized receptors, such as the T-cell receptor, and PAMPs. Resultant signalling cascades via PI3K/AKT increase mTOR signalling, in turn enhancing metabolic pathways typically associated with pro-inflammatory responses. AMPK, on the other hand, inhibits mTOR signalling and upregulates metabolic pathways associated with anti-inflammatory responses. Created using biorender.com

before undergoing controlled apoptosis. In neonates, there is a transient rise in neutrophil frequency shortly before birth, as measured by neutrophil-derived soluble FcRIII (CD16) levels in plasma of preterm and term infants. However, neutrophil frequency reduces again shortly after birth to levels below that of adults, contributing to diminished first responder function.[17,18] Neonatal neutrophils also have reduced functional capacity; their chemotaxis response is reduced through slower velocities and lower expression of the surface adhesion molecules L-selectin and Mac1, contributing to a near 50% reduction in neutrophil transmigration to the site of infection.[19,20] Lower expression of TLR4 and defective downstream signalling by myeloid differentiation primary response 88 (MyD88) and p38 MAPK from TLR4 and TLR2 impair neutrophil response.[21] High levels of adenosine in neonatal blood increase cyclic adenosine monophosphate (cAMP) leading to protein kinase A (PKA)-dependent/TLR-independent inhibition of tumour necrosis factor (TNF)α.[7] For neonatal neutrophils, some reports also show reduced capacity, or entire loss, of their primary effector functions of phagocytosis, neutrophilic oxidative burst and formation of neutrophil extracellular traps.[17,22] All of these are energetically demanding requiring high levels of glycolysis, the main metabolic pathway in neutrophils and, to a lesser extent, glutaminolysis to support them.[23,24] Inhibition of these metabolic pathways can entirely prevent effector functions of adult neutrophils.[23,24] For instance, NETosis is dependent on lactate derived from glycolysis in both NOX-dependent and independent pathways.[25] Whether changes in neonatal neutrophil metabolism are responsible for their decreased effector functions has not been elucidated but clearly warrants investigation. Ketone metabolism may be a key pathway to consider as ketone bodies provide an important fuel for neonates[26] and may be required for effector functions like NET formation.[27]

2.2 | Eosinophils

Elevated eosinophil counts in newborns have been reported with infection in preterm infants and have been linked to later atopy.[28] Little is known about the metabolic functions of eosinophils in general though experiments in adults show that eosinophils do use glycolytic metabolism.[29] However, eosinophil metabolic plasticity was demonstrated recently with both increased glucose-derived lactate production, when reactive oxygen species are inhibited, and generation of TCA intermediates from both glucose and glutamine, on cytokine stimulation.[30] Further work is needed to explore the role of metabolism in determining eosinophil function and to evaluate

whether there is any role for altered metabolism in eosinophilia in neonates.

2.3 | Type 2 innate lymphoid cells

Type 2 innate lymphoid cells (ILC2) are found at epithelial barriers such as in the lung and are a major source of IL-5 that can induce eosinophilia. ILC2 numbers rapidly increase in the lungs after birth, peaking at three times that of adults by day 10 but quickly decreasing to adult levels.[31] Among the ILC subsets, ILC2 is the most functionally mature compared with ILC1 and ILC3.[32] The alarmin IL-33, produced by epithelial cells, recruits eosinophils and ILC2 to the lungs and acts as a potent activator of ILC2.[31] Despite higher IL-33 levels in neonates, ILC2 activation is not as potent as in adults. The high levels of endogenous IL-33 in the neonatal lung are suggested to train the ILC2 response for a more efficient response to challenges in later life.[33] It is also worth noting that IL-33 signalling via ST2 under neonatal hypoxia is important for the expression of asthma-related genes.[34] Given that hypoxia-inducible factor-α strongly stimulates glycolysis, this suggests that a highly glycolytic programme of ILC2s in early life might have harmful effects.

In adults, ILC2 demonstrates the capacity to augment mitochondrial respiration above basal conditions when activated with IL-33. Arg1 is suggested as key to the function of ILC2 as inhibition leads to decreased production of effector cytokines such as IL-5 and IL-13 accompanied by significantly reduced glycolytic capacity.[35] Evidence indicating a glycolytic programme in ILC2 comes from experiments inhibiting glycolysis via PD1 which resulted in decreased expression of effector cytokines and reduced proliferation and cell survival.[36] Conflicting evidence using helminth infection in mouse models suggests fatty acid oxidation, rather than glycolysis, is necessary for expansion and cytokine production by ILC2.[37] Differing methods of ILC2 activation as well as differences between mouse and human responses may account for the conflicting results with further experiments needed to clarify this. Furthermore, given that neonates exhibit less potent responses than adults, despite increased IL-33 signalling and the emerging role of metabolism in ILC2 effector cytokine production, an investigation into the metabolic function of neonatal ILC2 is warranted.

2.4 | Monocytes/macrophages

Monocytes and macrophages perform key innate roles in clearing threats through phagocytosis and oxidative killing and contribute to adaptive responses through antigen presentation to T cells. Monocytes are better studied in neonates compared with macrophages as they can be isolated from blood. Studies of pattern recognition receptors are essentially restricted to TLRs, and neonates have similar expression levels compared with adults. However, neonatal monocytes have impaired TLR-mediated production of TNFα and other pro-inflammatory cytokines.[38] Reduced expression of TNFα has been associated with high levels of adenosine, a metabolite with immunomodulatory properties. Adenosine acts via the A3 adenosine receptor inducing production of the secondary messenger cAMP which inhibits TLR-mediated TNFα production.[7] Reduced MyD88 expression has also been implicated in failure to produce pro-inflammatory cytokines such as TNFα and IL-12p70. While limiting innate immune responses, this reduced capacity to produce key cytokines along with reduced expression of human leukocyte antigen (HLA)-DR and co-stimulatory molecules such as CD40 at the basal state also affect the ability to activate T cells.[39,40] However, after LPS treatment, expression of co-stimulatory molecules, MHC-II and CD80, does not differ significantly from adults.[41] Altered cellular metabolism is now emerging as a critical regulator of neonatal monocyte function, especially cytokine production. Whole-blood transcriptomic data suggest significantly increased activity in glucose and cholesterol metabolic pathways. Three glycolytic regulatory nodes—glucose transporter GLUT3 and glycolytic enzymes 6 phosphofructo-2-kinase and hexokinase 3—were upregulated in response to infection, but the cellular provenance of these was not identified.[42] Monocytes isolated from term and preterm umbilical cord blood have reduced glycolysis compared with adults. Glycolysis was required for cytokine production but not for phagocytosis which was unaffected by inhibition of glycolysis using 2-deoxy-D-glucose (2DG).[5] More recently, umbilical cord blood monocyte–derived macrophages were also shown to be broadly defective in glycolysis and to exhibit reduced oxidative phosphorylation compared with adult peripheral blood monocyte–derived macrophages.[43] IL-10-polarized umbilical cord blood–derived macrophages had adult-like levels of OXPHOS but reduced glycolysis. As for adenosine, circulating factors in umbilical cord blood seem to mediate this effect as treating adult peripheral blood–derived macrophages with cord blood plasma dramatically inhibited glycolysis. This was attributed to high expression of the S100 proteins S100A8/A9 in neonates.[43] Furthermore, cord blood monocyte–derived macrophages have reduced expression of co-stimulatory CD80 and CD86 and reduced capacity to stimulate T cells.[44]

Mammalian target of rapamycin (mTOR) and its downstream targets are essential for metabolic reprogramming of macrophages, with signalling through this pathway required for glycolytic reprogramming (Figure 1).[45] Surprisingly, mTOR transcripts are elevated in cord blood compared with adult blood–derived macrophages but total mTOR protein expression and mTOR phosphorylation are both reduced compared with adults. This disparity suggests post-transcriptional regulation of mTOR expression in neonates. Downstream targets of mTORC1, ribosomal protein s6 and eukaryotic translation initiation factor 4E-binding protein 1 also showed reduced expression and phosphorylation respectively compared with adults.[43]

The findings summarized above highlight that monocyte metabolism is critical to regulating the neonatal immune response. As variation in TLR-mediated cytokine production at birth has been linked to maternal health status and later development of allergic disease by the child,[46–48] identifying possible metabolic determinants of this

might provide insight into why this occurs and how it might be prevented or rectified. Therefore, links between metabolic control of innate cell function in neonates and immediate and long-term health outcomes deserve further investigation.

2.5 | Natural killer cells

Natural killer (NK) cells are crucial to the resolution of acute respiratory viral infections such as those caused by influenza or respiratory syncytial virus. Mature NK cells can be subdivided into two main subsets, a CD56bright CD16dim subset, which produces high amounts of inflammatory cytokines, and CD56dim CD16bright, which are a highly cytotoxic subset.[49] NK cell function is regulated by the balance of inhibitory and activating receptors expressed on the cell surface especially HLA-E binding members of the CD94/NKG2 family such as inhibitory NKG2A and stimulatory NKG2C and NKG2D.[50] Neonatal NK cells have been shown consistently to have reduced degranulation and cytotoxicity as well as reduced interferon (IFN)γ production.[51,52] This seems to be despite high degrees of similarity in phenotypic markers in comparison with adults, though upregulated expression of inhibitory NKG2A on cord blood CD56dim NK cells has been suggested to contribute to these reduced responses although this might be counterbalanced by upregulated NKG2D.[50,53]

Emerging data about the role of metabolism in NK cell function identify key pathways that should be investigated in the neonate. Murine NK cells rely on OXPHOS for homeostatic function and acute responses such as cytokine production, following short-term activation.[54] In human NK cells, early cytokine responses can occur independent of glucose.[55] Long-term activation of NK cells upregulates both OXPHOS and glycolysis to meet the metabolic demands of effector function [56] supported by increased expression of nutrient transporters such as GLUT1, CD98 and CD71 following stimulation.[56,57] Inhibition of OXPHOS limits NK cell IFNγ production and degranulation, whereas glycolytic inhibition impairs cytotoxic functions such as target cell killing and degranulation.[56,58] Regulatory factor mTORC1 is required for successful NK cell function—inhibition of its activity in murine NK cells prevents the upregulation of glycolysis needed for granzyme B and IFNγ production.[59] mTORC1 activity is also needed for NKG2D-mediated IFNγ production in human NK cell subsets.[57] Given the importance of metabolic reprogramming for the function of NK cells as summarized here, metabolic differences may be a key determinant of altered function of neonatal NK cells.

Given the possibility that metabolism may underpin changes seen in neonatal NK cell function, this leads to the question of what local environmental factors might regulate NK cell metabolism in early life. Various factors abundant in the neonatal circulation like adenosine and S100A8/A9 as discussed above might contribute to this along with TGFβ which is a candidate for regulating NK cell metabolism. TGFβ is abundant in utero and during early life[60–62] and is known to affect NK cell function including inhibition of cytotoxicity[63,64] and of CD16-induced IFNγ production through SMAD3 repression of transcription.[63] These inhibitory effects of TGFβ have been attributed to its role in inhibiting cellular metabolism. In short-term activation assays, IL-15-stimulated mouse and human NK cells treated with TGFβ had reduced mTOR signalling (primarily mTORC1), impaired cellular metabolism and reduced cytokine production.[65] While this also might be mTORC1 independent,[66] it seems that TGFβ inhibits metabolic pathways, such as glycolysis and OXPHOS, in human NK cells. As efforts are made to understand neonatal NK cell metabolism, parallel efforts should consider the role of TGFβ and similar immunomodulatory cytokines.

2.6 | Dendritic cells

Dendritic cells (DCs) have a critical role in educating and priming the adaptive immune response through integrating signals from the local microenvironment to modulate MHC, co-stimulatory molecules and cytokine expression, thereby orchestrating T-cell activation and effector function. The two major groups of DCs in humans are classical (cDCs) and plasmacytoid (pDCs). These are typically found in a ratio of around 3:1 in adult peripheral blood but occur at a ratio of 1:3 (cDCs to pDCs) in umbilical cord blood.[67] Neonatal monocyte–derived DCs have been described to favour the Th2-biased cytokine effector function of neonates (Figure 2).[68] This might reflect that, despite similar expression of TLRs to adult DCs, neonatal DCs have altered cytokine responses after TLR stimulation. Reduced secretion of the Th1-inducing cytokine IL-12p70 (Figure 2), along with decreased IFNγ secretion,[68] might underpin this. Upon stimulation with LPS, neonatal DCs also have attenuated expression of HLA-DR, CD86, CD80 and CD40 that might contribute to their reduced capacity to activate naïve cord blood T cells.[69] Similarly, in murine neonatal models, DCs have been shown to efficiently migrate and be capable of antigen presentation but fail to upregulate co-stimulatory molecules in response to IFNγ.[70]

In human adults, metabolic rewiring supports DC activation. During differentiation from monocytes and from bone marrow progenitor cells, DCs use OXPHOS as the primary means of energy production.[71] On activation, DCs switch to a glycolytic metabolism and inhibition of glycolysis decreases surface expression of IL-12p70, MHC class I and class II as well as co-stimulatory CD40 and CD86.[72] When looking at specific subsets of DCs, pDCs use both glycolysis and glutaminolysis fuelled OXPHOS to support activation with inhibition of these pathways resulting in decreased expression of HLA-DR, CD80, CD86 and IFNα.[73,74] However, cDCs rely more exclusively on glycolysis which is crucial for CD40 and IL-12 expression. In contrast to pDCs, TLR stimulation reduces OXPHOS in cDCs.[74] Given the known down regulation of co-stimulatory molecules and reduced cytokine production in neonatal DCs and their established link with metabolic reprogramming, investigation of underlying metabolic differences in neonates is warranted including comparison to other subsets, especially monocytes, to look for common denominators.

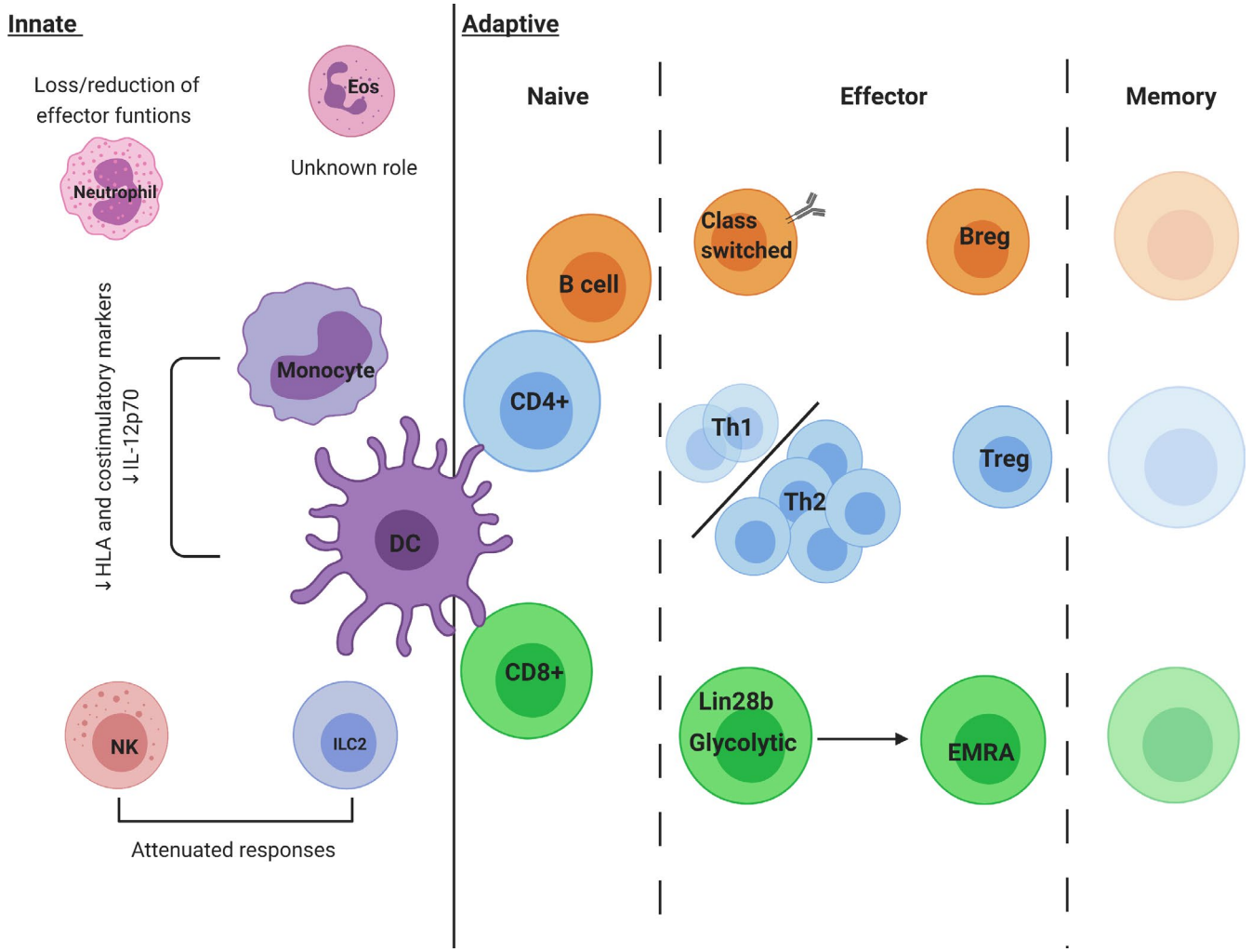

FIGURE 2 Immune responses of neonates. Innate responses of neonates are characterized by attenuated responses and reduced pro-inflammatory phenotype with loss of some effector functions entirely. Adaptive responses are dominated by naïve cell subsets and generally less inflammatory responses, preferring the generation of regulatory cells. Created using biorender.com

2.7 | Myeloid-derived suppressor cells

Myeloid-derived suppressor cells (MDSC) are a heterogeneous population of early myeloid-derived progenitor cells which share an immature state and the ability to suppress T-cell activation. While dichotomized into monocytic MDSC (M-MDSC) and granulocytic MDSC (G-MDSC) subsets, in humans these phenotypes are poorly established. However, general consensus defines these subsets as CD11b+ CD14+ HLA-DR-/low CD15- and CD11b+ CD14- CD15+, respectively.[75] In adults, MDSC are typically associated with the pro-inflammatory environment of tumours and other chronic inflammatory conditions. As such, MDSC have been studied primarily for their immune suppressive role in the tumour microenvironment where they potently suppress T-cell expansion and effector functions.

Recently in humans, the suppressive capacity of M-MDSC has been linked to specific metabolic adaptations. Compared with monocytes, the dicarbonyl metabolite methylglyoxal is 30-fold higher in M-MDSC inhibiting glycolysis in these cells.[76] M-MDSC also transfer cytoplasmic methylglyoxal to T cells in a contact-dependent manner and inhibit T-cell metabolic reprogramming to suppress their expansion and effector functions.[76] Methylglyoxal also selectively depletes L-arginine and L-glutamine,[76] which are both amino acids required for T-cell function and might explain reports of depletion of L-arginine by MDSC.[77] Other studies have demonstrated that fatty acid uptake and FAO mediated by STAT3/STAT5 and lipid uptake by CD36 are required for generation of highly suppressive MDSC in the tumour microenvironment.[78,79]

Compared with healthy adults, MDSC are transiently increased at birth with cord blood levels equivalent to those of cancer patients.[80] MDSC frequency in neonates positively correlates with birth weight.[81] MDSC from healthy weight neonates have greater suppressive capacity than those from very low weight neonates, potentially indicating a link between nutrient availability and MDSC function. Interestingly, breastmilk lactoferrin is key to generating M-MDSC and G-MDSC able to suppress neonatal T cells; however, the same effect is not observed in adult MDSC.[80,82] Additionally,

lactoferrin treatment induced potent bacterial killing function in neonatal MDSC[80] demonstrating potential roles beyond immune suppression. In mouse models of neonatal necrotizing enterocolitis, lactoferrin-treated MDSC are currently being investigated as a potential therapeutic due to their ability to suppress T-cell activation and inflammation.[80,81]

3 | ADAPTIVE IMMUNE RESPONSES

The adaptive immune system of neonates is commonly described as immature. While memory T-cell populations can be identified within the foetus, possibly arising from the foetal intestine,[83,84] nearly all neonatal T and B lymphocytes display markers of antigen inexperienced naïve cells.[17,83,84] Recent studies have also revealed the failure of human and mouse neonatal lymphocytes to differentiate into long-lived memory cells and their reduced secondary responses upon antigen re-exposure.[85–87]

3.1 | B cells

Neonatal B lymphocytes are almost exclusively antigen inexperienced naïve B cells. Neonates have reduced class-switched antibody production, with almost no IgG or IgA antibody production on activation compared with adults which have been suggested to contribute to greater susceptibility to infection of neonates.[88] Depending on the method of activation used, neonatal B cells exhibit both enhanced and reduced proliferation, leading researchers to conclude that while reduction in B-cell proliferation may be observed in some settings; neonatal B cells do not have a general defect in proliferation.[88] However, survival of neonatal B cells across various subsets is decreased with most methods of activation. While much less is known about the immunometabolic features of B cells compared with other immune cells, we do know that unlike most other immune cells the upregulation of glycolysis does not appear to be crucial for their activation, proliferation or differentiation, despite increased import of glucose after activation.[89] Rather, B cells appear to rely on OXPHOS to meet the demands of activation and proliferation, likely supported by glutaminolysis rather than glucose metabolism.[89] However, glucose does seem to be required for class switch recombination which is known to be impaired in neonates.[88,89] When assessing B-cell subsets for enzymes related to glycolytic and oxidative metabolic pathways, naïve B cells were found to have the lowest expression across all enzymes examined. Memory subsets had higher expression of metabolic enzymes while plasma cells had the highest expression of all enzymes.[90] A recently identified subset of B cells, now termed regulatory B cells (Bregs), characterized as CD25hi CD38hi performs important regulatory functions, promoting tolerance during pregnancy.[91] Bregs suppress the immune system through direct cell-to-cell signalling via CD80/CD86, IL-10 and adenosine generation by the purine ecto-enzyme CD73. Bregs preferentially downregulate IFNγ production along with the differentiation of Th1 and Th17 cells but not regulatory T cells (Tregs).[92,93] Increased frequency of Bregs in cord blood has been suggested to play a role in the altered immune response of neonates, with their suppressive role postulated to be primarily through production of IL-10 and co-stimulatory CD80/CD86 signalling to T cells.[94,95] Adenosine production is unlikely to be a mechanism used by neonatal Bregs for immune suppression as CD73 is markedly and selectively downregulated.[96]

Along with the observations summarized for other cell types above, impaired glycolysis might be a common mechanism of altered neonatal immune cell function although the upstream determinants of this might differ by cell type.

3.2 | T cells

T cells are highly diverse and perform a multitude of specialized functions contributing to and orchestrating the immune response. Here, we will focus primarily on CD4+ (T helper), CD8+ (cytotoxic T cells) and CD4+FoxP3+ regulatory T cells.

3.2.1 | CD4+ T helper cells

While the neonate is not devoid of memory CD4+ T cells, as already noted, neonatal CD4+ T cells are predominantly naïve (CD45RA+ CCR7+) and overwhelmingly display characteristics of recent thymic emigrants (RTEs) (Figure 2).[97] Neonatal CD4+ T cells that do not receive signals from IL-7 or other common γ chain cytokines are more susceptible to apoptosis leading to a high cell turnover rate.[97,98] IL-7 promotes the survival and maturation of CD4+ RTEs without inducing differentiation,[99] and this is supported by higher telomerase expression in neonates, which prevents shortening of the telomeres in this early stage of development.[100] This high IL-7-induced proliferative capacity is suggested to help maintain diversity in the T-cell repertoire [101] and, at least in mice, IL-7-induced neonatal T-cell proliferation is linked to higher STAT5 activation, a signalling pathway shared with humans.[102] The metabolic requirements to support rapid T-cell proliferation in neonates are unknown however, in adults aerobic glycolysis and OXPHOS drive proliferation and differentiation of CD4+ T cells.[103] We have shown a requirement for STAT5 signalling in very early adult naïve T-cell activation. Inhibiting STAT5 in naïve but not effector or central memory CD4+ T cells resulted in reduced glutamine-dependent anaplerosis and loss of IL-2 production,[104] highlighting the critical role of metabolic reprogramming and the use of glutamine in the very earliest stages of T-cell activation in the periphery.

As mentioned earlier, CD4+ T-cell cytokine responses are considered to be Th2 skewed in neonates (Figure 2), in part due to altered functions of cells such as DCs. Additional to antigen-presenting cells influencing neonate T-cell phenotype through diminished IL-12-p70 (as discussed previously), monocyte-derived pro-inflammatory cytokines have been shown to suppress IL-2 production inducing non-classic Th2 cells.[46] However, characteristic

interleukin production of naïve CD4+ T cells also has been linked to epigenetic modification. Hypermethylation at CpG and non-CpG sites occurs within and adjacent to the IFNγ promoter region in neonatal naive CD4+ T cells and is accompanied by markedly reduced IFNγ production by neonates upon T-cell activation.[105] Although reduced IFNγ production is widely reported in neonatal αβ T cells, it is, however, worth noting that neonatal γδ T cells do not exhibit the same defect.[106]

In mice, neonates are poised for a Th2 type response. They exhibit pre-existing CpG hypomethylation at the CNS-1 locus when resting and CpG methylation at the CNS-1 locus remains lower than in adult mice after 5 days, under Th2-polarizing conditions.[107] In human neonates, the Th2 locus is extensively remodelled, with hypomethylation and permissive histone modifications selectively in Th2 cells cultured under Th2-polarizing conditions.[108] A novel subset of neonatal naïve CD31+ CD4+ T cells storing an unglycosylated isoform of IL-4, not present in adults, has also been described, however, any unique function of these cells has not yet been identified.[109] The relative balance of cytokines has implications in early life programming of later immune function and allergic disease risk in particular.[3] Greater expression of IL-13 at birth, along with other epigenetic variations in metabolic genes such as *RPTOR*, *PIK3D* and *MAPK1*, has been linked with subsequent susceptibility to atopic disease in childhood.[108,110] Reduced IFNγ at this time is a long-recognized hallmark of allergic disease risk.[111] Therefore, there is much value in elucidating the immunometabolic regulation of T-cell effector cytokine production at this stage of development to provide mechanistic insight and therapeutic targets for mitigating non-communicable disease risk.

T-cell effector function has been linked closely with metabolism in various studies[16] and may have a role to play in skewed T-cell responses of neonates. Glycolysis is a specific requirement for IFNγ production in humans and in mice through post-transcriptional regulation by the glycolysis enzyme glyceraldehyde phosphate dehydrogenase (GAPDH).[112–114] In vitro and in vivo blockade of glycolysis is also known to reduce expression of the Th2 cytokines IL-4 and IL-13 and whether GAPDH has a role to play here too is still unknown.[115] Furthermore, TGFβ signalling significantly inhibits mitochondrial complex V resulting in decreased ATP production and impaired IFNγ production in human CD4+ T cells.[116] Some work has begun to explore how altered mitochondrial respiration in neonates might contribute to altered T-cell activity. So far, mitochondrial mass of neonatal CD4+ T cells has been shown to be lower than adults and accompanied by reduced levels of ATP.[117,118] However, on activation of T cells from preterm infants, higher calcium flux into the mitochondria than adults is observed along with increased ERK phosphorylation suggesting enhanced signalling for metabolic reprogramming.[119] Finally, nutritional regulation of T-cell development has been shown in mice where oligosaccharide diets were found to have a role in regulating neonatal Th1 type responses in respiratory syncytial virus infection models.[120] Such approaches might offer therapeutic strategies should immunometabolic signatures be found associated with disease outcomes.

3.2.2 | CD8+ cytotoxic T cells

A number of features of neonatal CD8+ T cells have been associated with increased susceptibility of neonates to infection. CD8+ T-cell responses in neonates are described as innate-like, with reduced cytotoxic capability and increased expression of anti-microbial protein transcripts.[121] Exploration of the epigenomic landscape has revealed changes associated with lower TCR signalling and cytotoxicity but higher expression of genes involved in cell cycle.[121] In line with these findings, it is widely reported that neonatal CD8+ T cells are highly proliferative through homeostatic proliferation.[100,122,123] This highly proliferative programme extends into activation.[124] Neonatal CD8+ T cells divide more than their adult counterparts over the first three days of activation with the cells entering division sooner after initial activation and each division then happening faster in neonates. Differentiation, measured by CD62L and Ly6C expression, also occurs faster in neonates (Figure 2) yet they become terminally differentiated, shown by CD127 downregulation and KLRG1 upregulation, and fail to differentiate into long-lived memory cells (Figure 2), producing weak CD8+ T-cell expansion on secondary challenge.[124,125] Rapid proliferation and differentiation in neonates are suggested to impair the development of memory CD8+ T cells.[124] Studies in mice suggest a role for the inhibitor of let-7 microRNA, lin28b, in maintaining a neonate-like phenotype by CD8+ T cells (Figure 2).[126]

To support their highly proliferative phenotype, murine neonatal CD8+ T cells preferentially rely on glycolysis.[85] Indeed, neonatal mice exhibit higher glycolytic metabolism than adults, a response attributable to functional programming by lin28b (Figure 2). High glycolytic metabolism was also linked directly to the inability of neonates to form memory populations with pharmacological inhibition of glycolysis with 2DG able to restore formation of memory cells.[85] In comparison with mice, our understanding of human neonatal CD8+ T-cell metabolism is not as well developed and what little evidence there is contrasts. For example, human umbilical cord blood CD8+ T cells have a propensity to differentiate into non-classical T cytotoxic (Tc)2 cells, when activated and this was associated with a decrease in glycolysis and an increase in fatty acid metabolism of Tc2 cells.[127] Much more work is needed to properly understand the role metabolism has to play in the fate of human neonatal CD8+ T cells.

3.2.3 | Regulatory T cells

Regulatory T cells (Tregs) are crucial in preventing immune dysregulation and promoting peripheral tolerance. The subset defined by expression of the transcription factor forkhead box (FOX)p3 will be the focus here. Tregs have emerged as critical to the success of pregnancy playing a vital role in maternal tolerance of the foetus and immunoregulation more generally at the foetal-maternal interface.[128] Furthermore, maternal factors such as allergy have been suggested to reduce Treg suppressive function in the offspring.[129] IL-2R-STAT5 and TGFβ-SMAD signalling promote Treg differentiation through recruitment to CNS2 and CNS1 of the FOXP3 locus, respectively.[130,131]

Tregs have high mitochondrial mass, relying on OXPHOS via mitochondrial oxidation of pyruvate and lipids upon activation, unlike conventional T cells that primarily rely on aerobic glycolysis.[132,133] While proliferation of Tregs is fuelled primarily through OXPHOS, glycolysis appears to be crucial to the suppressor function of Tregs. Enolase-1, a glycolytic enzyme, functions as a regulator of conserved non-coding sequence (CNS)2, preventing the transcription of a FOXP3 splice isoform containing exon-2 (FOXP3-E2).[134] Exogenous metabolites might also affect the stability of Tregs. Tregs have increased cell surface expression of ecto-nucleotidases CD39 and CD73, which convert ATP into immunosuppressive adenosine.[135] In neonatal T cells, CD39 expression is highest in activated Tregs, demonstrating the importance of their immunomodulatory function; the frequency of this population of Tregs correlates inversely with clinical severity of sepsis in neonates.[136] Intriguingly, neonatal CD4+FOXP3- T cells display a natural propensity to differentiate into Tregs[98] (Figure 2) likely reflecting suboptimal stimulation via TCR.[137] Given the role metabolism has in determining T-cell fate, an intrinsic metabolic programme in naïve neonatal CD4+ cells linked to early post-TCR activation events might favour the preferential generation of Tregs contributing to the development of immune tolerance at this time.

3.3 | Outlook

Immunometabolism is now a well-established field enabling significant advances in our understanding of human immune cell fate and function. However, to date, little progress has been made in applying this to the understanding of human neonatal immune responses. Some initial work is revealing fundamental differences in cellular metabolism likely underpinning well-described phenotypic and functional differences across the life course. However, caution must be taken when the focus remains on umbilical cord blood collected at the transition from intrauterine to extrauterine life and the changes in fuel demand and provision that occur around this time. We also must be mindful that neonates do not simply lack in comparison with adults and alternative fuel substrates (e.g. ketone bodies as above) and higher levels of exogenous metabolites (e.g. adenosine as above) might drive unique phenotypes at this stage of development. Much more work is needed especially extending observations beyond umbilical cord blood to samples collected in the days, weeks and months after birth. Such work is limited by sample volumes and will need to focus on single-cell functional genomic approaches linking transcriptional and translational regulation of the mechanisms at play accompanying these with emerging flow cytometry-based approaches to the study of immunometabolism such as SCENITH.[138] Future work should establish the metabolic underpinnings of neonatal immune cell function and explore how the unique energy demands, immune requirements and rapid development of neonates have led to this highly evolved, age-appropriate phenotype. Understanding immunometabolism in neonates is an exciting field of research potentially harbouring new ways of combatting many diseases by providing untapped insight into diverse functional roles of the immune system.

IRB STATEMENT

All of our work involving samples from human participants is reviewed and approved by a Health Research Authority or University research ethics committee with samples and data collected with informed consent of the donor.

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CONFLICT OF INTEREST

None to declare.

AUTHOR CONTRIBUTION

Sean R Holm: Writing-original draft (lead). **Benjamin James Jenkins:** Writing-original draft (supporting); Writing-review & editing (supporting). **April Rees:** Writing-review & editing (supporting). **James G Cronin:** Supervision (supporting); Writing-review & editing (supporting). **Nicholas Jones:** Supervision (supporting); Writing-review & editing (supporting). **Catherine A Thornton:** Supervision (lead); Writing-original draft (supporting); Writing-review & editing (lead).

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