

Review article

Neonatal immunity: how well has it grown up?

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Several factors are conspiring to focus attention on neonatal immunology. First, the dogma that neonatal exposure to antigen results in tolerance rather than immunity has been challenged. Second, there is a clinical imperative; the development of effective neonatal vaccines is likely to be extremely important in reducing infant morbidity and mortality, and the successful use of cord blood stem cells for bone marrow reconstitution calls for a better understanding of the regulation of responsiveness in neonatal lymphocytes. Third, recent evidence suggests that exposure to high levels of antigen during early life might contribute to the increasing prevalence of allergic diseases.

Neonatal B cells

Although neonates can mount immune responses, they are still relatively immunodeficient (Table 1). This could reflect inherent, differences between adult and neonatal B and/ or T cells or some aspect of the neonatal environment that modifies their responses. The responsiveness of neonatal T cells to antigen has been reviewed recently¹.

Table 1. Characteristics of murine neonatal antibody responses¹

Antigen type	Adults	Neonates
T-independent type 1 (TI-1)	++	++
T-independent type 2 (TI-2)	++	+
T-dependent (TD)		
Isotype switching	+++	Weak
Affinity maturation	+++	Poor
Heterogeneity	+++	Restricted

Neonatal B cells are phenotypically and functionally distinct from their adult counterparts (Table 2) although the precise differences in signaling pathways between adult and neonatal B cells remain to be fully elucidated^{2,3}.

Ligation of the B-cell receptor (BCR), which induces activation and proliferation in mature adult B cells, fails to have this effect on neonatal B cells. Rather, it transduces negative signals that

inhibit subsequent responses to antigen or mitogen and make B cells prone to apoptosis^{4,5}

Table 2. Functional and biochemical differences in signal transduction following B-cell receptor ligation on mature and immature B cells^{2,3}.

Response	Neonatal B cells	Mature B cells
Proliferation	-	+
Apoptosis	+	-
Ca ²⁺ flux	+	+
Phosphoinositide hydrolysis	-	+
FYN expression	-	+
FGFR I expression	-	+
FOS induction	-	+
EGR I induction	-	+
MYC induction	-	+
Cyclin E induction	-	+

T cell responses

Although murine studies demonstrate a bias towards T helper-2 (Th2) type T-cell responses, this is less evident in humans. Human neonatal T cells generally produce less interleukin 2 (IL-2), IFN-gamma and IL-4 than adult T cells, possibly because of the memory status of significant numbers of adult T cells. In studies that compare the responses of purified CD4 'naive' T cells from neonates with those from adults, similar cytokine profiles have been observed⁶.

Neonatal and adult naive T cells do not differ in their ability to produce high levels of Th1-type cytokines, or to develop into Th2 effectors upon repetitive stimulation. Therefore, if there is a bias in human neonatal T cells towards Th2-type cytokine production, it cannot be attributed to neonatal T-cell immaturity alone, but must also involve contributions from other immature or dysregulated accessory cells, particularly dendritic cells (DCs) and B cells.

The responsiveness of human neonatal T cells to individual cytokines differs markedly from that of adult T cells. Neonatal human T cells proliferate in the presence of IL-4⁷, and recently, IL-7 has been shown to be a potent inducer of proliferation in neonatal, but not adult, T cells. IL-7 also acts as a survival factor for naive T cells⁸, maintaining their

viability and expansion for significantly longer than adult naive T cells.

T-B interactions in neonates

B cells of neonates show a selective inability to upregulate expression of MHC class-II following BCR ligation⁹. Because antigenic peptides are targeted to newly synthesized MHC class II¹⁰, the failure of neonatal B cells to induce its hyperexpression of class-II must result in a reduced array of peptide-loaded MHC at their surface. This implies that neonatal B cells are likely to deliver weaker signals to T cells via their T-cell receptors (TCR). These might be insufficient to reach the threshold for T-cell activation, and fail to lead to CD40 ligand (CD40L) induction and the establishment of a productive T-B interaction. This would be compounded by the diminished capacity of neonatal T cells to upregulate CD40L expression¹¹.

Differences in antigen handling by neonatal B cells could also contribute to the reported bias of neonatal responses towards those of the Th2 type. Experiments that examine Th polarization under various conditions of antigen presentation provide evidence that low MHC-peptide density, as probably occurs in neonates, favours priming of Th2-type CD4+ cells. Furthermore, BCR ligation does not induce the expression of B7.2 (CD86) on neonatal B-cells. Neonatal B-cells are thus unlikely to deliver the costimulatory signals to T cells via CD28, which are pivotal in defining the outcome of TCR ligation. Neonatal B cells might thus be expected to be inefficient or ineffective APCs and, where they do work, to preferentially induce Th2 helper cells and direct Th0 cells towards the secretion of cytokines that characterize Th2 cells. The inability of neonatal B cells to upregulate key molecules that are involved in their interaction with T cells might also create conditions in which antigen encounter results in tolerance -with T cells receiving a 'weak' signal-1 (low density peptide-loaded MHC class-II) in the absence of costimulatory signal-2 being rendered anergic or even deleted. The failure of BCR ligation to induce B7.2 expression on neonatal B cells could also contribute to the induction of tolerance by enhancing their elimination¹².

Cytokines -a possible role

As indicated earlier, part of the explanation for poor neonatal responses might lie in the immaturity of the individual rather than of the B

cells *per se*. In addition to the actions of LT-alpha, LT-beta and TNF that are mentioned above, recent experiments have suggested other cytokines that might affect responses in early life.

IL-10 is made by B1 cells¹³, which are more common in neonates than in adults, and by neonatal T cells, which secrete high levels of this cytokine. IL-10 downregulates the appearance of MHC class II complexes at the surface of monocytes and inhibits their ability to act as APC¹⁴. It does not, however, have similar effects on B cells¹³. High levels of IL-10 in neonates might thus tend to favour B cells that act as APC, with the consequences described above.

Transforming growth factor-beta1 (TGF-beta1) also downregulates constitutive MHC class II expression by human peripheral blood mononuclear cells (presumably including both B cells and monocytes). Intriguingly, a genetic modification that has resulted in expression of TGF-beta1 in the pancreatic islets of non-obese diabetic (NOD) mice protects them from diabetes, apparently by altering the APC preference of the NOD T cells from B cells to macrophages¹⁵.

Why do neonates fail to make antibody responses to polysaccharide antigens?

Human neonates are particularly deficient in their responses to bacterial capsular polysaccharides, resulting in significant infant morbidity and mortality from infectious diseases. Antibody responses to complex polysaccharides (TI-2 antigens) are assumed to be T-cell independent. Experiments in mice that lack B1 cells have shown clearly that these cells are essential for anti-polysaccharide responses. Although neonates have relatively large numbers of CD5+ B cells, they are only responsive later in ontogeny. This could be due to the lack of the appropriate microenvironment. Responses to this class of antigen are predominantly of IgM and IgG3 isotype. The ability of these antigens to induce memory formation (which is T cell-dependent) is limited¹⁶. Recent improvements in our understanding of the cellular and molecular events that underpin memory generation provide the basis for a more detailed investigation of these observations. The need for this is strengthened by reports that show that TI-2 antigens can influence B-cell development positively¹⁷ and induces phenotypic changes that could account for the priming effect.

Some polysaccharide antigens can activate the complement cascade by the alternative pathway and; this appears to be important for their immunogenicity. The role of complement might be to focus antigen on marginal zone B cells or APCs, or to

augment B-cell responsiveness by allowing co-ligation of the BCR and CD21 on B cells. Human neonatal cord-blood B cells are CD21⁺, although the molecule might be present at lower levels than on adult B cells¹⁸. CD21 appears to be functional on neonatal B cells, as they proliferate in response to Epstein-Barr virus (EBV). Importantly, neonatal human B cells that are unresponsive to type 4-pneumococcal polysaccharide can be induced to respond when their CD21 molecules are ligated with anti-CD21 mAb¹⁹.

Human neonates are, however, relatively deficient in C3, which only reaches adult levels at around one year of age²⁰, at about the same time as polysaccharide responses develop. Class switching is severely affected in C3-depleted mice; memory responses are ablated by complement depletion and C3-knockout mice exhibit severe defects in primary and secondary responses to TD antigens and in GC formation²¹. Low C3 levels in neonates might therefore contribute to the low degree of isotype switching and defective generation of memory that are seen in their responses to TD antigens and to their deficient response to polysaccharides. These observations suggest that it would be interesting to examine the immunogenicity of both TD- and TI-antigen-C3 conjugates in neonates.

Concluding remarks

In conclusion, there are several mechanisms that might operate to produce the relative immunodeficiency that is seen in human neonates. Some might reflect developmentally regulated changes in B-cell signaling that could influence the establishment of effective interaction between T and B cells, which are necessary for secondary responses. Defective B-cell signaling might account for tilting of the immunity-tolerance balance in neonates and the reported bias of their T-cell responses towards those of Th2 type. Recent evidence also suggests that B cells are required to generate signals for the orderly development of lymphoid tissues, and that regulation of their ability to do so might be influential in determining the acquisition of responsiveness to polysaccharide antigens and the ability to generate memory responses.

The estimated 1.8 million deaths annually from infection during the first year of life, predominantly in developing countries, is a daunting challenge. Immunology can play a major role in reducing this unacceptable level of mortality through effective neonatal and/or

maternal vaccination. A full understanding of the neonatal immune system and its capacity to respond effectively to the diversity of antigens that will be required for protection during early life is therefore a priority.

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