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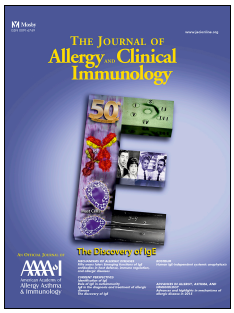

The neonatal window of opportunity – early priming for life

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Paradigms and Perspectives

The neonatal window of opportunity – early priming for life

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List of abbreviations

BCR	B-cell receptor
COPD	chronic obstructive pulmonary disease
EPEC	enteropathogenic Escherichia coli
HDM	house dust mite
IgD	immunoglobulin D
IgM	immunoglobulin M
IL	interleukin
iNKT	invariant natural killer T cells
LPS	lipopolysaccharide
NCD	non-communicable diseases
NEC	necrotizing enterocolitis
NF-κB	nuclear factor kappa-light-chain-enhancer of activated B-cells
NKT cells	natural killer T cells
RNA	ribonucleic acid
TLR	toll-like receptor
WHO	World Health Organization

Abstract

The concept of the neonatal window of opportunity assigns the early postnatal period a critical role for life-long host-microbial and immune homeostasis. It is supported by epidemiological evidence that links postnatal environmental exposure with disease susceptibility and mechanisms in the neonate host that facilitate the postnatal transposition, establish a stable microbiome and promote immune maturation. During the conference on "The neonatal window of opportunity – early priming for life", postnatal microbiome and immune maturation, epidemiological evidence and fundamental mechanisms were discussed to identify new targets for future preventive and interventional measures.

From December 5-7, 2016, the Herrenhausen Conference “The neonatal window of opportunity – early priming for life” took place at Hannover, Germany, sponsored by the Volkswagen Foundation. The concept of the “neonatal window of opportunity”, i.e. a critical non-redundant time frame in a newborn’s life during which environmental factors drive immune and tissue maturation and influence the susceptibility to immune-mediated and other diseases in adult life, was discussed.

Please note: An extended version of the manuscript can be found in the supplement.

Non-communicable diseases (NCD) as the grand challenge for prevention

Our body is exposed continuously to many danger signals. An effective immune system is essential in order to protect and repair. Unbalanced immune reactivity seems to play a key role in non-communicable diseases (NCDs) such as chronic pulmonary disease (COPD), allergies, asthma, diabetes, cancer, and cardiovascular diseases as well as obesity (Johan Garssen). Prevention is the grand challenge, since causal therapies for NCDs are still not available and prevalences and incidences are currently on the rise.

Maternal/fetal/neonatal interaction

The first “window of opportunity” in life is defined as the pre- or perinatal period. Particularly in this period, microbial factors have a strong impact on the development of immune responses. This includes the birth place and mode of delivery, breastfeeding, medication (e.g. antibiotic use), introduction of solid foods, but also the exposure to siblings and pets (John Penders). Challenging the immune tolerance of neonatal mice, germ-free mice exhibited an exaggerated allergic response when exposed to allergens, as compared to conventionally raised mice. Once their airway microbiome had formed after 2 post-natal weeks, the mice were protected against this over-shooting allergic inflammation (Benjamin Marsland) [1]. However, oral antigen administration in early life, even in the presence of protective breast milk-derived factors, does not guarantee the induction of oral tolerance and the prevention of allergic disease. This implies the necessity to identify limiting factors for oral tolerance induction in early life (Valérie Verhasselt) [2].

Healthy nutrition during pregnancy and early life represents a key factor for proper immune development. Human milk still remains the gold standard (Johan Garssen).

Development of immune tolerance in the neonatal period

This goes hand in hand with the ontogeny of the enteric mucosal tissue and its influence on the establishment of the enteric microbiota and long-term host-microbial homeostasis as well as infection susceptibility. Age-dependently expressed features of the innate and adaptive immune system that contribute to the postnatal acquisition of mucosal homeostasis and the particular susceptibility of the neonate host to infection (Mathias Hornef) [3].

Early events and new insights into mechanisms

Streptococcus pneumonia is a leading cause of bacterial infection of the upper and lower respiratory tract in children. This pathogen spreads to its host following acquisition of very few organisms that quickly proliferate and dominate the niche in the upper respiratory tract. This work established a **new paradigm for understanding pathogen transmission** and the initial establishment of the colonizing microbiota in newborns (Jeffrey Weiser). This is also influenced by the exposure to maternal microbiota metabolites in utero and in early life during lactation that prepares the newborn for colonization with its own microbiota and sets the baseline for a regulated immune system (Kathy McCoy) [4]. Important novel mechanisms are summarized in figure 1. Neonates are often highly susceptible to intestinal pathogens. An exception is seen after infection of murine neonates with *Yersinia enterocolitica*. Here, multiple innate and adaptive components including (surprisingly) **CD8+ T lymphocytes cells**, participate in protective immunity (Becky Adkins). **Natural killer T (NKT) cells** function as a kind of checkpoint regulating the crosstalk between the microbiota and the immune system. iNKT cells are resident cells of the gut mucosa that establish their niche during early life when they home to and expand during a restricted period of neonatal life. Studies performed in germ-free mice suggest a critical neonatal time period for the development and proliferation of these immune cells (Richard Blumberg) [5]. **γδ cells** in mice represent a first line of defense with respect to both early in life during T-cell development and ontogeny as well as in terms of mucosal tissue localization. The human neonate γδ TCR repertoire contains considerable frequencies of innate “public” TCR-δ sequences matching conserved microbial structure (Immo Prinz). Symbiotic bacteria control the selection processes that determine the antibody repertoire of **naïve B-cells** in neonatal mice. Interestingly, this training event appears to be particularly influential during a narrow window of opportunity after birth as commensal bacteria exposure of adult germ-free mice showed no effect on the

positive selection events (Duane Wesemann). Tissue specific **memory T cells** are established as non-circulating resident memory T cells at various tissue sites in response to microbial stimulation (Donna Farber) [6]. Necrotizing enterocolitis (NEC) is the leading cause of death from gastrointestinal disease in premature infants and develops after an exuberant proinflammatory response to the colonizing microbiota and this requires activation of the lipopolysaccharide (LPS) receptor, **Toll like receptor (TLR) 4** (David Hackam) [7].

Role of environmental microbes

Epidemiological studies show a strong correlation between early microbial exposure to allergens and a reduced risk of hypersensitivity leading to asthma or hay fever later in life (Erika von Mutius) [8]. The diversity and duration of microbial exposure early in life is important for the development of tolerogenic immune functions. In this regard, mild proinflammatory signals including IL-6 play a critical role to orchestrate early activation signals (Harald Renz) [9].

New methods

Stem cell-derived organoids represent a new three-dimensional model system of the gut epithelium that allows the study of organ development, immune homeostasis and tissue regeneration. Moreover, epithelial stem cell organoids now also open the possibilities to study host-microbe-interaction (Sina Bartfeld).

System biology approaches applied to the global transcriptome generated from the RNA isolated from a few drops of blood from pre-term babies have helped to reveal how regulatory mechanisms determine the set-point for immune homeostasis in early life by integrating specific immune and metabolic pathways that inter-patient differences can be overcome and uncovered a novel immune-metabolic axis that accurately predicts sepsis in neonates and infants. These studies lay the foundation for future translation of host pathways in advancing diagnostic, prognostic, and therapeutic strategies for neonatal sepsis (Peter Ghazal) [10].

Conclusion

We are only starting to appreciate the critical impact of the postnatal period for lifelong health and disease susceptibility. Recent discoveries provide the proof of principle, characterize the critical time window(s) and identify underlying mechanisms. However, a number of important questions remain: The cause-effect relationship between microbial exposure and health needs to be established and the mechanisms operating during this “window of opportunity” need to be elucidated. This must consequently lead to effective primary prevention strategies. Future research will further deepen our understanding and extend from animal models to human studies in order to develop interventional strategies to foster immune homeostasis and prevent the development of diseases.

Figure legend

Figure 1 New mechanisms of microbe-immune interaction. For details please see text.

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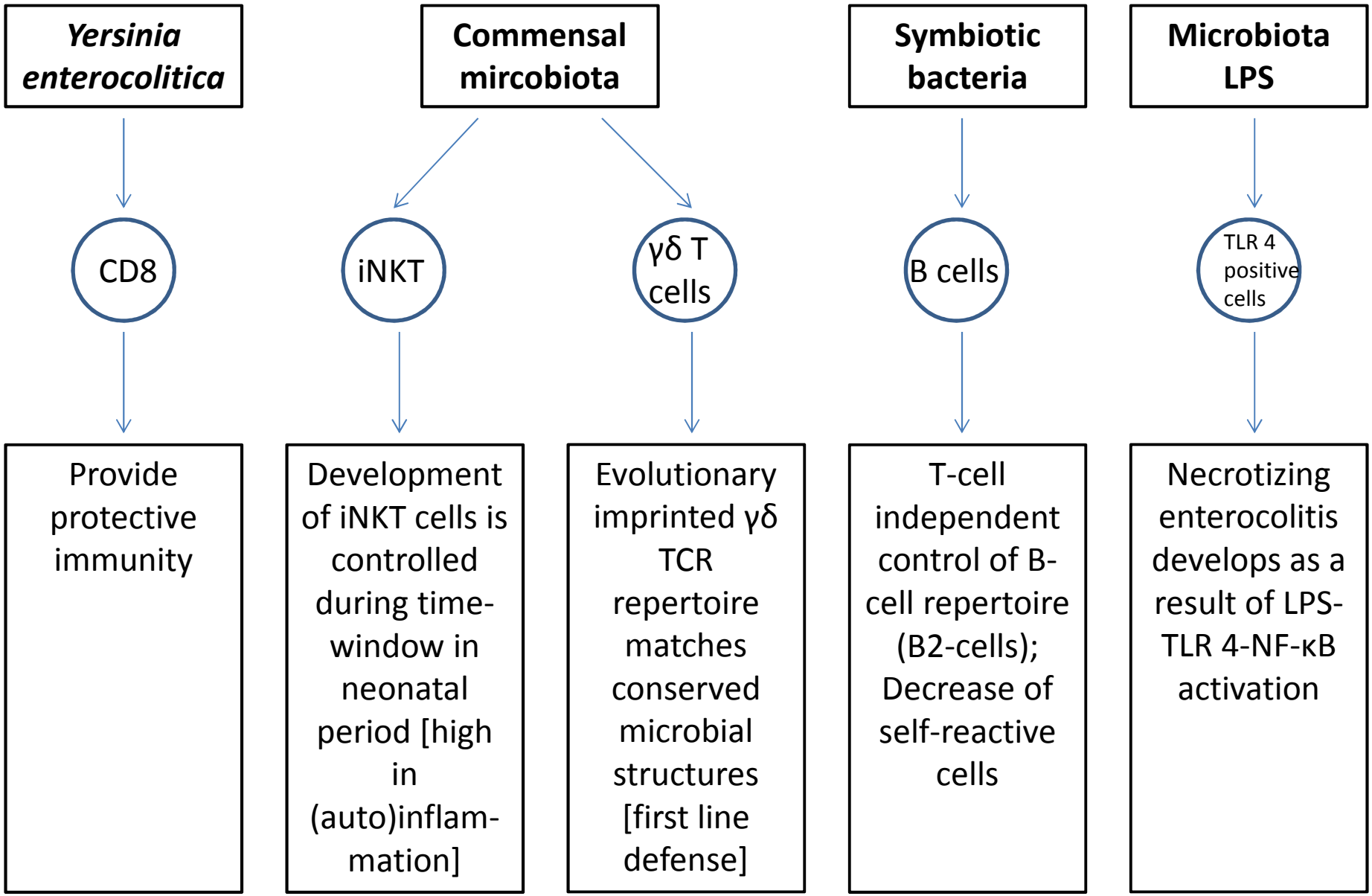

Figure 1