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Technologies for assessing vaccine responses in the very young

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Many vaccines are administered to young children in order to prevent infectious diseases early in life. At the same time, most of these vaccines are not developed specifically with the immune system of young children in mind and our understanding of how newborn immune systems differ from adult counterparts is incomplete. The main reason for this lack of understanding stems from the ethical and logistical difficulties in obtaining samples from young children as well as the challenges associated with the small volume samples available. Here I review some recent developments made in this field and discuss their implications for studying vaccine responses in young children and developing better vaccines, tailored to this important population of susceptible individuals in the future.

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Introduction

Human immune systems are highly variable among individuals, yet rather stable within individuals over time in the absence of acute infection or inflammatory stimuli [1]. At the same time, over longer time scales, there are clear age-associated differences and characteristic differences that distinguish immune system composition and function in the very young, middle age, and elderly [2]. In fact, some recent studies indicate that the age of an individual can be predicted from the changes in its immune system [3], and some factors driving such age-associated changes, such as chronic cytomegalovirus have been reported [4]. Overall, human immune systems are shaped by a combination of heritable and non-heritable influences with the latter typically explaining the majority of the overall variance in a population [5–7]. A general pattern is that the innate branch of immunity is more strongly influenced by heritable traits than the adaptive branch of the immune system which is more amenable to environmental cues [8,9]. All of these advances have relied on a new form of immune system investigation, inspired by technologies and theories from the field of systems biology. These systems immunology approaches allow for more complete analyses of the many proteins and cell populations constituting the immune system, as well as the various transcriptional programs and activation states within these cells. All of these layers of information can be captured simultaneously in a given blood sample. In ideal cases such analyses can reveal co-regulated features in the system and generate hypotheses to be pursued in mechanistic follow-up experiments. Also, these types of studies capture the inter-individual variation better than traditional reductionist studies, and uncover patterns of coregulated features associated with age, sex and other metainformation.

Systems immunology

Modern measurements from individual immune cells, such as high-dimensional mass cytometry, flow cytometry, sorting, and single-cell mRNA-sequencing, all enable very careful investigations to be made of the cells present in a sample. Such measurements also enable more data to be extracted from a given volume of blood. Combined with high-dimensional plasma protein analyses and metabolomic profiles, nearly all the relevant components of blood human immune systems can be simultaneously captured and taken into account [10,11]. Another important advancement has been the ability of assessing functional responses by individual immune cells using either mass cytometry [12], microchip secretomic assays [13], and single-cell RNA-sequencing [14•]. When studying vaccine responses, it is imperative to know the specificity of cells responding to distinguish vaccine-specific cells from other activated cells. To this end, the use of multiplex sets of metal-tagged MHC-multimers loaded with peptide antigens allow for deep interrogations into vaccine responding cells [15]. Also, sequencing of clonal receptor genes in bulk immune cells is informative, and grouping genes identified into specificity groups can identify all clones simultaneously responding to a vaccine [16•] Sequencing of variable receptor genes can also be performed in single cells allowing researchers to reconstruct the B-cell evolution during vaccine responses [17]. For individual T-cells, knowing TCR sequences in a given HLA-context can even allow you to identify the antigen recognized by screening reactivity against large

peptide pools and so on [18]. As sequencing-based methods evolve, the new frontier is to combine various layers of measurements in individual immune cells, as illustrated by simultaneous measurements of T-cell receptor genes and epigenomic cell states [19•], and the simultaneous measurement of transcriptional programs and protein expression in single immune cells during an immune response [20]. With these exciting advances vaccine responses are now becoming increasingly interpretable. Vaccines also serve as important perturbations that can uncover regulatory pathways in the human immune system [21–25], and this will be important for us to better understand the particular features of the immune system in young children in the future.

Ethical and logistical challenges in studying young children

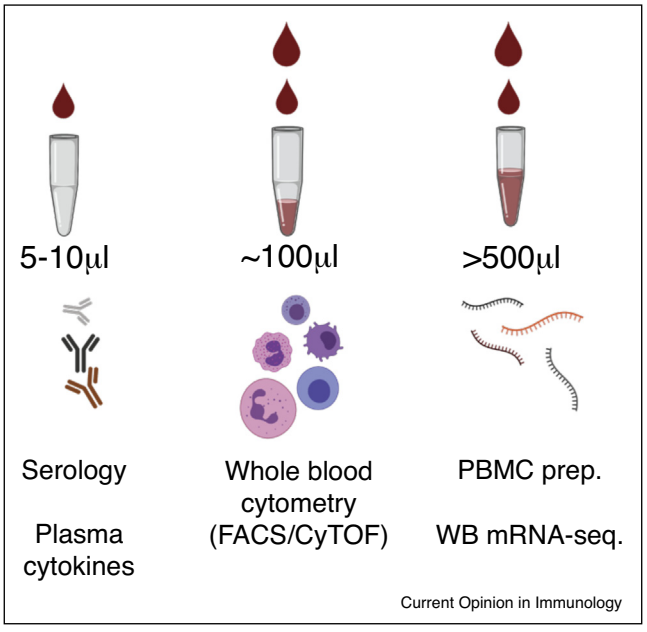
Studies in children are always more challenging from an ethical point of view as small children are unable to consent themselves and are instead represented by their legal guardians. There are also specific technical and logistical challenges in studying newborn and young children given. In small children it is estimated that the total blood volume is around 85−100 ml/kg of body weight [26]. In a small preterm child weighing half a kilogram or less, this means that the entire blood volume could be as low as 40 mL. Such extremely preterm or growth restricted newborns always require intensive care early in life and repeated blood sampling to guide this care. Therefore, it is not unusual that within a few weeks after birth, a large portion for the entire blood volume has been collected for clinical chemistry and been replaced with transfusions of erythrocyte concentrates. Experimental research studies performed in such extremely sensitive infants is obviously of secondary importance as compared to the blood samples need for clinical care purposes and all samples collected should be as small and as carefully considered as possible. This is not only true in this extreme example of the very preterm or growth restricted infants, but also illustrates the challenges with blood sampling in any newborn child. According to the World Health Association, WHO, recommends no more than 3 mL/kg to be sampled during the first day of life in sick newborns and a blood draw under 5% of total blood volume during the first 24 hours after birth, and less than 10% of total blood volume during the first eight weeks of life is recommended as minimal risk to the newborn child [26]. Since this volume is the combined volume used for research and clinical purposes, it is key to weight the benefit and importance of clinical sampling against the need for research studies. With new and improved sampling methods and experimental techniques, we can now perform more studies also in highly susceptible and fragile individuals such as these preterm newborns.

Studying immune responses in young children

Systems-immunology type methods that provide high-dimensional measurements in a given sample are ideal for small volume samples because they allow all cell types, many proteins and genes to be measured simultaneously [18]. A recent study from our group recently illustrated this by profiling newborn immune systems, longitudinally in 100 newborn children at birth, and during the first few weeks of life, by profiling nearly 300 plasma proteins using Olink assays, and all immune cell populations by mass cytometry [27•]. This study was enabled by recent advances in preserving blood samples directly as whole blood since complex sample procedure systems based on density gradient-separation are not easy to perform in the context of clinical blood sampling in an intensive care unit.

These measurements required only 100 μl of blood and provided a first systems-level profile of the developing newborn immune system in preterm and term-delivered children [27•]. In a more recent advancement of this small volume sampling, we have taken advantage of improved methods for collecting the sample from the patients, such as nearly pain-free capillary blood sampling using microneedles [28•] and capillary forces that allow for a similar blood volume of about 100 μl to be profiled and a

Figure 1



Examples of analyses possible with different volumes of blood. The analyses of antibody titers and plasma cytokines can often be performed with only a few microliters of plasma or serum. Whole-blood preservation methods avoid cell loss during preparation and allow full cytometry experiments using as little as 100 μl of blood. PBMC-preparation by density gradient separation and genome-wide mRNA-sequencing of whole blood cells preserved in stabilizer solution often require about 500 μl of blood.

complete mass cytometry mediate immune cell profile generated [29]. This will prove increasingly important for studies in small children, as the pain associated with traditional sampling methods is one major hurdle for patient inclusion and return visits for research. The move from peripheral blood mononuclear cells to whole blood as the cell material to use for cell-based assays also minimizes the technical variation introduced by manual sample handling and consequently increases the biological sources of variance that can be detected [30•].

The need for blood volumes differs significantly between different assays. Serological testing such as vaccine-induced antibody measurements can often be performed using only a few microliters of serum or plasma (Figure 1). Cell-based assays do not always require a lot of cells, but it is easy to lose a lot of cells during processing, such as cell staining, washing and cell sorting. By minimizing these losses [31] and preserving whole blood cells immediately upon collection [30•], cell-based assays are now possible using as little as 100 μl of blood as starting material [27•,29] (Figure 1). For separation and storage of viable immune cells, density gradient separation is normally required, and this typically requires about 500 μl of blood as starting material. Similarly, in our experience to get good mRNA-sequencing libraries from whole-blood samples preserved by PaxGene tubes or other methods, we normally use about 500 μl, although this can likely be decreased with further optimization (Figure 1).

Conflict of interest

P.B is a co-founder and shareholder of Cytodelics AB.

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