Challenges in infant immunity: implications for responses to infection and vaccines

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Infections in infants continue to be an important cause of morbidity and mortality worldwide. Understanding the immune mechanisms that operate in infants is necessary for the development of new approaches to improve the health of infants around the world.

Despite advances in medicine and technology, approximately 4 million children under the age of 6 months die each year worldwide because of infection, a rate of ~450 deaths per hour[1]. The cost of hospitalization for infected infants (0–12 months of age) in the United States alone is estimated at $690 million annually, with 40,000–50,000 infant deaths per year[2,3]. The rising incidence of premature birth (less than 32 weeks of gestation) contributes to a substantial percentage of neonatal deaths globally (**Fig. 1a**). In addition, although the immune system of an infant born at term (after 37 weeks of gestation) affords some protection against infectious disease, impaired responses to a range of pathogens and vaccines result in susceptibility to severe disease in a substantial number of infants. For example, pneumonia caused by polysaccharide-encapsulated bacteria (such as *Streptococcus pneumoniae* and *Haemophilus influenzae* type b (Hib)) is a leading cause of death for children under the age of 5 and is the most serious of the lower respiratory infections in this age group[4] (**Fig. 1b**). Although vaccines are available to protect against infection by these organisms, studies of vaccinated infants have shown that immunity wanes around 6–9 months after vaccination, and multiple booster shots are needed to sustain immune responses and maintain immunological memory in this population[4]. Moreover, there are no vaccines for infants against certain childhood pathogens, such as respiratory syncytial virus (RSV), that often cause very serious infections in infants[5]. Therefore, better understanding of immune system development in infants and the unique immune characteristics at this developmental stage might help in the design of better interventions to improve the health of this vulnerable population.

In 2000, the United Nations outlined eight development goals, called Millennium Development Goals, as a vision for the future to improve health, education, opportunities and the environment around the world. One goal is to diminish by two thirds the mortality rate of children under 5 years of age by 2015. This goal includes decreasing the infant mortality rate and increasing the proportion of 1-year-old children who are immunized against measles (http://www.mdgmonitor.org/goal4.cfm). The global perspective of the these goals is highly relevant to the integral mission of the National Institute of Allergy and Infectious Diseases (NIAID; http://www.niaid.nih.gov/about/whoWeAre/Documents/niaidstrategicplan2008.pdf), a component of the National Institutes of Health: to understand the mechanisms involved in immune regulation and immune protection in people, including infants, and to apply this knowledge to better treat and ultimately prevent infectious, immunologic and allergic diseases that afflict millions in the United States and around the world. The interests and mission of the Bill and Melinda Gates Foundation complement those of NIAID. This foundation focuses on developing ways to fight and prevent diseases in people, including infants, who live in resource-limited countries. The foundation works on implementing and scaling up proven approaches, as well as on promoting better resources and policies in resource-limited settings.

The NIAID convened a workshop 3–4 June 2010 to discuss the subject of infant immunity. This workshop was organized by the Division of Allergy, Immunology and Transplantation of the NIAID and was cosponsored by the Bill and Melinda Gates Foundation. The goals of the workshop were to assess the present base of scientific knowledge on infant immunity; to identify research gaps and key issues in immune mechanisms in the infant that affect the generation and maintenance of protective immunity to infection; and to foster collaborations among investigators studying infectious disease pathogenesis, vaccine design and development, and immune mechanisms in infants. This commentary focuses on various aspects of the infant

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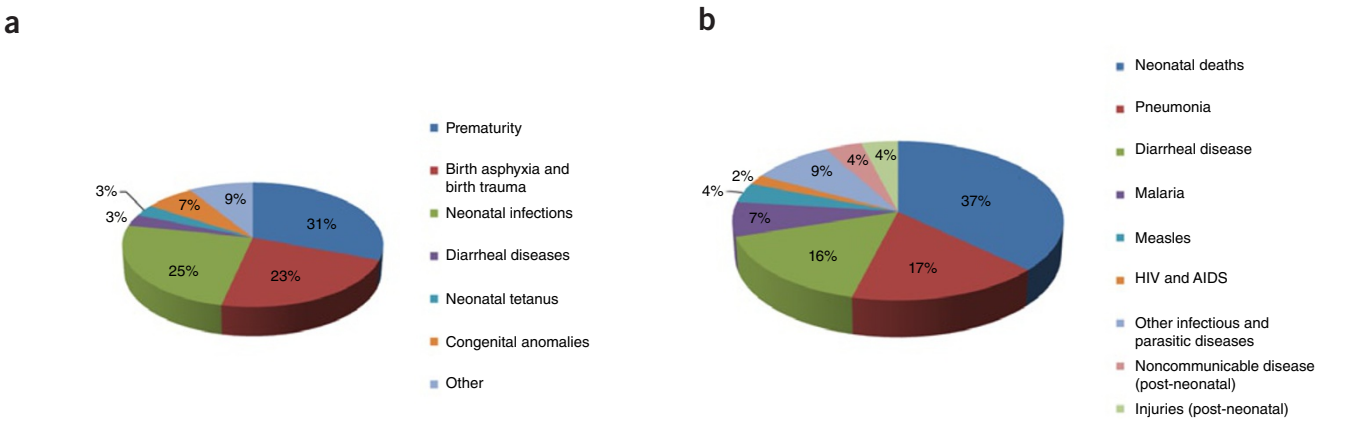

Figure 1 Worldwide mortality in neonates and children under 5 years of age. (**a**) Worldwide causes of neonatal deaths (birth to 1 month of age). Premature infants are those born at less than 32 weeks of gestational age. (**b**) Worldwide causes of death in children under the age of 5. Figure adapted from ref. 25.

immune response and highlights workshop discussions of key issues that influence infant responses to infection and vaccines.

Immune system development in infants

Infants have limited exposure to antigens *in utero* to induce adaptive immunity. Therefore, they are thought to be heavily dependent on their innate immune system for protection against infections. Toll-like receptors (TLRs) participate in the recognition of microbial pathogens relevant to infants, such as group B *Streptococcus*, *Listeria monocytogenes* and RSV[6]. Tobias Kollmann (Vancouver, Canada) examined TLR-mediated cytokine production by mononuclear cells and demonstrated differences among blood samples from neonates (0–1 month), toddlers and adults. He showed that infant blood monocytes produce less interferon-α (IFN-α), IFN-γ and interleukin 12 subunit p70 (IL-12p70) than do cells obtained from adults. However, production of these cytokines rapidly increases between birth and 1 or 2 years of age. In contrast, infant cells show a greater capacity to produce IL-10 and strong IL-17-producing helper T cell (T_H17 cell)–promoting function in response to TLR stimulation by producing IL-6 and IL-23. Furthermore, individual infant cells are less able than adult cells to produce multiple cytokines simultaneously in response to TLR agonists; that is, infant cells are less polyfunctional. The predominance of a T_H17-like pattern combined with considerable IL-10 production may contribute to diminished T helper type 1 (T_H1) responses, resulting in greater susceptibility to intracellular infections and diminished vaccine responses during infancy. Understanding the molecular mechanisms that regulate the pattern of cytokine expression by innate cells in infants will be necessary for the development of protective measures, such as vaccines, for this population.

The adaptive immune system consists of cell-mediated and antibody-mediated responses, and the development of adaptive immune cells in early life is another understudied area of research. Low numbers of memory-effector B cells ($CD27^+$) and effector-memory T cells ($CD45RA^-CD45RO^+$) are detected during early infancy, as expected given the limited exposure to antigen at this stage. David Lewis (Stanford, California, USA) showed that recent thymic emigrants (RTEs), which are T cells recently produced by the thymus, are present in a large proportion in the periphery of human infants[7], and these RTEs are impaired in their acquisition of T_H1 function[7]. That characteristic may contribute to an infant's vulnerability to infection with *Mycobacterium tuberculosis* and other intracellular pathogens. RTEs also predominate in infant mice[8] and have impaired T_H1 function, which indicates that this may be a general feature of infant immunity in mammals. Large numbers of transitional B cells (with high expression of CD5, CD10, CD24 and CD38), which have been produced recently in the marrow and emigrated to the blood, are found during early infancy. As with RTEs, transitional B cells are more functionally limited than are more mature naive B cells. Thus, the predominance of transitional B cells and T cells may have a role in the vulnerability of infants to infection. Further studies that address the function and phenotype of such transitional cell populations would define and cast light on their role in the function of total B cells and T cells in infants.

Studies of cell-mediated responses suggest that infants are able to mount T cell responses in most circumstances. However, the quantity and quality of the response may differ from that in adults. For example, $CD4^+$ T cell responses, but not $CD8^+$ T cell responses, develop more slowly in infants than in adults after primary infection with cytomegalovirus or herpes simplex virus[9]. In addition, responses to some vaccines, such as vaccines for hepatitis B virus and oral poliovirus vaccine, are diminished in T_H1 activity and biased toward T_H2 function[10]. Although there is evidence for the presence of many cytokines in response to vaccines, good correlates of protective cellular immunity that are informative have not yet been defined. The mechanisms underlying the functionally distinct responses of infant T cells are not well understood but are probably multifactorial.

First, infants have a dominant anti-inflammatory cytokine profile that seems to be induced during fetal life[10]. Joseph McCune (San Francisco, California) demonstrated that in the *in utero* environment, $CD4^+CD25^{hi}$ $Foxp3^+$ regulatory T cells dominate the fetal circulation, suppressing reactivity to non-inherited maternal antigens[11] and possibly promoting a generally suppressive environment. Second, emerging data obtained in studies of mice and humans indicate that distinct epigenetic profiles and processes may have a major role. Hypomethylation of cytokine loci contributes to the expression of cytokine genes, whereas hypermethylation of such loci contributes to the silencing of cytokine genes. The T_H2 locus is hypomethylated in both human and mouse infants, relative to its methylation in adults[12,13], which corresponds to the propensity for T_H2-polarizing cytokine responses in infants. Moreover, infants and adults show differences in the expression of microRNAs that regulate the transcription of cytokine genes[14]. Therefore, it would be helpful to understand the role of epigenetic events in the silencing or expression of cytokine genes and their contribution to immune development in infants.

The B cell arm of adaptive immunity also commonly seems to be compromised in early life. Infant antibody responses are typically of shorter duration, have a delayed onset, differ in the distribution of immunoglobulin G (IgG) isotypes (lower titers of IgG2) and are of lower affinity than are adult responses[15]. In addition, naive B cells from neonatal mice and humans have lower expression of the cell surface receptors CD21, CD40, CD80 and CD86, and both neonatal mice and humans show defects in germinal center formation that resolve at approximately 3 weeks of age in mice and 4 months of age in humans[15]. Furthermore, although primary IgG responses to vaccines can be elicited within 2 months after birth, the persistence of protective antibody titers is poor. Because antibodies are key components of protective vaccine responses, greater understanding of B cell function during infancy will be important for the development of effective infant vaccines.

The developing microbial ecosystem in various mucosal sites is important for the development of the immune system in infants. The infant gastrointestinal tract is colonized soon after birth by a variety of commensal bacteria that influence the development of the immune response both in the gut and systemically. The mucosal immune system in the gut must avoid adverse immune responses to dietary antigens and commensals in the new microbial ecosystem while remaining able to mount an effector response to pathogenic organisms. Josef Neu (Gainesville, Florida, USA) noted that dysfunction of the intestinal barrier in infants can result in a variety of diseases, including sepsis, inflammatory diseases and atopic disease. Infant mice have low numbers of $CD4^+$ T_H17 cells in the lamina propria that may be related to delayed post-birth colonization by the intestinal commensal bacteria that induce the development of such cells[16]. Becky Adkins (Miami, Florida) demonstrated that robust T_H17 responses can develop earlier in the presence of pathogenic bacteria. *In vitro* restimulation of mesenteric lymph nodes from mice initially infected as neonates (1–10 days of age) with the enteric pathogen *Yersinia enterocolitica* results in large amounts of both IL-17 and IFN-γ. These results suggest that neonatal $CD4^+$ T cells are able to produce inflammatory cytokines that confer protection against gastrointestinal infection, although the systemic immune responses to antigens in infants tend to be more anti-inflammatory in nature (as described above). Further studies are needed to determine the mechanisms whereby effector versus tolerant responses are generated in the developing infant gut and how mucosal immune responses are different from overall systemic immunity in infants.

Environmental and genetic factors

The developing immune system is influenced by many factors, including intrinsic genetic differences, disease states and environmental exposures. For example, infants with primary immunodeficiency (PID) are at greater risk of infection and may develop poor responses to vaccines. Infants with PID can also develop active infection after vaccination with a live vaccine, including those for varicella zoster virus; measles, mumps and rubella; oral polio virus; Bacillus Calmette-Guérin (BCG); and rotavirus[17]. Louise Markert (Durham, North Carolina, USA) noted that methods now being used to diagnose PID result in the identification of toddlers with PID after infections have become evident but do not permit the early diagnosis of infants before the development of infectious complications. The implementation of testing to identify patients with PID early in life before the administration of live vaccines would protect such children from vaccine-induced infections. One example of such a test is the use of PCR to detect T cell antigen receptor excision circles as a marker for RTEs. An additional challenge in PID is the development of guidelines based on the number or function of T lymphocytes and B lymphocytes for use of live vaccines in children with known PID.

Another population of immunosuppressed infants is those receiving organ transplants. They have lower incidence of rejection than do older children[18], which may be due to a predominance of circulating regulatory T cells or the development of immune tolerance to allogenic antigens. Stuart Sweet (St. Louis, Missouri) noted that infants receiving immunosuppressive regimens have a compromised T cell response that may diminish the induction of protective immunity to infection and vaccines. Leveraging knowledge of the development of regulatory T cells to delineate the mechanisms by which tolerance to allografts develops in infants will provide better understanding of their role in maintaining allografts and may lead to lowering of the doses or shortening of treatment periods of immunosuppressive regimens.

Genetic and environmental factors in early life may contribute to the development of allergy and asthma in childhood. Children who grow up in poor urban neighborhoods have higher rates of allergies and asthma. James Gern (Madison, Wisconsin, USA) discussed results from the Urban Environment and Childhood Asthma study in which cytokine responses of cord blood mononuclear cells to a variety of stimuli were compared with season of birth, parental characteristics, *in utero* stressors and fetal growth. Season of birth was most closely associated with variations in cytokine responses, particularly IFN-α and IFN-γ responses. Ethnicity, birth weight and maternal asthma were also found to influence cytokine response patterns at birth. Finally, babies born to parents with allergies or asthma generally had diminished cytokine responses to a variety of innate and polyclonal stimuli. In addition, other studies have shown that RSV infection is a leading cause of bronchiolitis and pneumonia in infants between 1 and 3 months of age and may predispose children to wheezing later in life[5]. Because of the high prevalence of childhood asthma and the severity of this disease in urban children, expanding the knowledge of factors that contribute to this disease will have far-reaching implications for pediatric health.

Sophie Moore (The Gambia, Africa) discussed possible links between nutritional status and immune competence in an infant's life. She noted that undernutrition during gestation may result in a smaller thymus that in turn may affect the development of T cells in the infant. Her study in The Gambia showed that during periods of low food intake, the infant thymus is smaller, corresponding to fewer RTEs containing signal-joint T cell antigen receptor excision circles, a measure of thymic T cell output. Weaker T cell responses resulting from a contracted and less diverse T cell repertoire may impair infant immune responses to vaccines and infection. The mechanisms that link poor nutrition during early life to longer term defects in immune function require further study.

Vaccine strategies for infants

Infants are immunized with many vaccines to protect them from infections such as measles and mumps. B cell responses and antibody production are critical for the development of protective immunity induced by most vaccines, although data suggest that T cell immunity may be a better correlate of protection for some viral pathogens. Hayley Gans (Stanford, California, USA) discussed how limited infant B cell responses to vaccines may be due in part to diminished T cell help, immature antigen presentation, the presence of maternal antibodies, lower IgG responses to protein and particularly to polysaccharide antigens, and less antibody persistence. For example, passively acquired maternal antibodies impede the effectiveness of antibody-mediated protection elicited by measles vaccines[19]. For this reason, in settings in which measles exposure during infancy is uncommon, the vaccine for measles is typically given at 12 months of age or beyond. However, immunization is given at 9 months of age in high-risk settings so balance can be achieved between the need for early protection provided by T cell immunity induced under these conditions and the less-effective antibody response

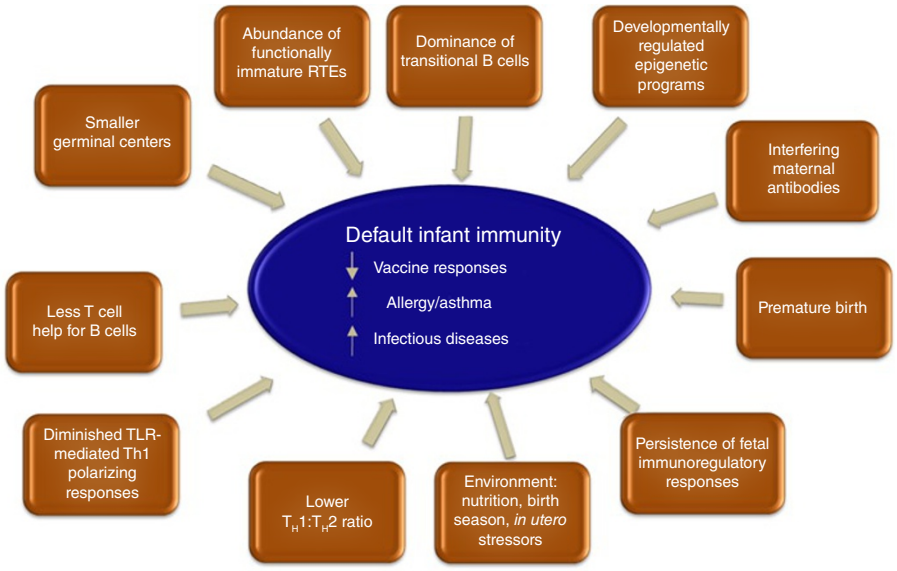

Figure 2 Present understanding of the factors that contribute to the state of an infant's immune system.

that develops at this age as a consequence of residual passively acquired maternal antibodies. The effects of a prime-boost phenomenon are important in infant vaccination strategies for the establishment of immunity early that can then be boosted after further exposure. Thus, identifying the epitope specificities of such maternal antibodies could be used to inform vaccine development to overcome this blockade.

Claire-Anne Siegrist (Geneva) discussed practices now in use in which infants receive multiple vaccinations and demonstrated that interactions between vaccine components can affect the overall response. Vaccines administered in infants to prevent bacterial infections include a vaccine for diphtheria–tetanus–acellular pertussis, a vaccine for Hib, and a pneumococcal conjugate vaccine. In a published study[20], a vaccine for acellular pertussis was given at 2–5 days of age along with a vaccine for hepatitis B virus, followed by a vaccine for diphtheria–tetanus–acellular pertussis, hepatitis B virus and inactivated poliovirus plus a vaccine for Hib at 2, 4 and 6 months of age. Early exposure to the vaccine for acellular pertussis elicited more rapid antibody responses to pertussis antigen than did vaccination beginning at 2 months. However, antibody responses to the vaccines for Hib and hepatitis B virus were diminished, reflecting vaccine interference. As the magnitude and quality of immune responses vary with the type of vaccine, Martin Ota (The Gambia, Africa) also emphasized the importance of carefully evaluating interactions between vaccines before integrating new vaccines with existing ones. Thus, understanding the mechanisms underlying vaccine interference and reassessing infant immunization schedules will allow the development of more effective vaccine strategies for infants.

There is also a need to identify alternative routes, dosages, combinations of vaccines, and adjuvants for improving vaccine efficacy in infants. In the United States, there is at present only one licensed adjuvant, alum (aluminum salts), for use in infants. Other adjuvant candidates are in various stages of development, but there is limited information about their mechanisms of action in infants. Ofer Levy (Boston, Massachusetts) focused on the need for adjuvants in infant vaccines to improve T_H1-polarizing responses at birth, a practical point of healthcare contact worldwide. Antigen-presenting cells from neonatal human cord blood demonstrate impaired T_H1 responses to many stimuli, including most TLR agonists, which reflects in part the inhibitory effect of adenosine in neonatal blood plasma. TLR8 agonists are refractory to adenosine inhibition and induce the production of adult concentrations of tumor necrosis factor and IL-1β in the cord and peripheral blood of rhesus macaques and human infants. Combined TLR7-TLR8 agonists also activate plasmacytoid dendritic cells via TLR7, inducing robust production of IFN-α and upregulation of CD40. Thus, engaging certain TLRs may be a potential avenue for the development of candidate vaccine adjuvants for use in vaccines for neonates and infants. Volker Gerdts (Saskatchewan, Canada) described the use of an infant porcine model to evaluate a pertussis vaccine with an adjuvant combination of polyphosphazenes, CpG (TLR9) and host-defense peptides to facilitate delivery, recruitment and immune stimulation, respectively. Pigs were protected from infection, as demonstrated by minimal lung lesions and bacterial colonization. Studies of potential vaccine adjuvants should be closely aligned with studies of protective immune correlates. For example, the development of effective vaccines for RSV infection is a priority, but progress has been slow because of incomplete understanding of the mechanisms responsible for immune protection against RSV versus disease potentiation of RSV. Thus, elucidating the immune responses that are required for the protection of young infants against detrimental infections would provide a basis for developing more effective vaccines and protective measures in this population.

Although vaccines have an important role in protecting infants from infectious diseases, other modes of protection may also be important. These may include antibiotics and passive antibodies. For example, prophylactic antibiotics are given to mothers to protect infants from infection with group B *Streptococcus* during birth. However, this approach will need to be weighed against the possibility of developing resistance or modifying the developing intestinal microbiome, which contributes to the development of the mucosal immune system. Hence, it would be beneficial to explore the role of passive antibodies as a mode of protection in infants. One way to provide passive protection is via maternal vaccination that is not detrimental to the developing fetus. Carol Baker (Houston, Texas) cited studies in which maternal IgG specific for capsular polysaccharides from group B *Streptococcus* protected infants from invasive infection. In a double-blind controlled study in which type III capsular polysaccharide conjugated to tetanus toxoid was administered to pregnant women at 30–32 weeks of gestation, the vaccine was safe and elicited a robust specific IgG antibody response that was transferred through the placenta and detected in infant serum at 2 months of age. Additional studies are needed to identify bacterial pathogens that would be amenable to maternal vaccination (such as *Bordetella pertussis*), which could lead to the prevention of disease in the infant after exposure and consequently lower the dose of antibiotics that infants receive as prophylaxis. Another mode of protection includes the administration of intravenous immunoglobulin (IVIg) or monoclonal antibodies such as palivizumab to protect against RSV infection. Ruth Karron (Baltimore, Maryland) noted that the prophylactic use of palivizumab lowers the risk of lower respiratory infection in infants; however, the associated costs are high. It is also important to determine the immune responses required for protection of infants against infection of lower respiratory tract by RSV.

Preterm birth can result from a variety of causes, including maternal stress, environmental triggers, genetic abnormalities and infection. Infectious agents have the ability to promote inflammatory responses in the mother and fetus, and most preterm births can be correlated with the presence of infectious agents and inflammation. Although infection-mediated preterm birth is substantial, the underlying mechanisms remain unknown. Premature infants are, in turn, at risk for infections (such as sepsis), especially while receiving care in hospital intensive care units. James Wynn (Durham, North Carolina, USA) discussed the role of IVIg in preventing and treating infections in infants with very low birth weight. Meta-analyses of prophylactic IVIg (polyclonal or pathogen specific) have not shown an associated decrease in the rate at which preterm infants develop infections. In addition, the clinical data for very preterm infants treated with polyclonal IVIg are limited. The immunological effects seen in older pediatric and adult patients may not be directly applicable to infants. Thus, further studies to determine the immune mechanisms underlying therapeutic efficacy of IVIg in infants with very low birth weight would be advantageous and may inform the use of this protective measure in this highly susceptible population.

Infant immunity in the developing world

As noted above, one of the Millennium Development Goals is to diminish the mortality of children under 5 years of age by two thirds by 2015. Substantial progress is being made toward this goal in all parts of the world, although rates remain high in many regions, including sub-Saharan Africa, where nearly 50% of such deaths occur[2]. Both social and biomedical changes, including better delivery of existing vaccines and the development of new and more affordable vaccines, are needed to accelerate progress toward this goal.

The administration of vaccines in early infancy is a cost-effective strategy for protecting infants from infectious disease in the developing world. However, many factors may limit vaccine efficacy in such resource-limited settings. As noted above, poor nutrition *in utero* and in the infant is associated with a smaller thymus and may impede T cell development and function, although additional evidence and mechanistic insights from well-controlled human studies are needed to determine the extent to which such differences are causally related to a higher risk for infection and impaired responses to vaccines. Chronic environmental enteropathy, a condition associated with poor sanitation and persistent repeated exposure to enteric pathogens, is associated with impaired responses to enterally administered vaccines; however, the causal relationships between these and other factors, including impaired nutritional status, remain poorly understood and must be addressed to inform knowledge-based interventions[21].

There is also a high prevalence of coinfections in developing countries, which may hinder vaccine efficacy. Coinfection with human immunodeficiency virus and malaria or cytomegalovirus represents a complication for pregnant women and fetal development. In such women, malaria can restrict fetal growth, result in preterm delivery and low birth weight in newborns, diminish the transfer of maternal immunity, and dampen responses to other infectious diseases such as *Streptococcus pneumoniae*, tetanus and measles. For example, titers of antibody to tetanus were 48% lower in newborns whose mothers had placental malaria[22]. Sarah Rowland-Jones (Oxford, UK) reported a strong correlation between the viral load of human immunodeficiency virus and the viral load of cytomegalovirus in coinfected infants, together with greater mortality, which suggests that early cytomegalovirus infection acts as a cofactor in rapid disease progression in African infants infected with human immunodeficiency virus.

BCG is given to children at birth in developing countries to protect them from tuberculosis. In a study of 6,000 infants to determine correlates of risk of tuberculosis disease after BCG vaccination at birth, Willem Hanekom (Cape Town, South Africa) demonstrated a lack of correlation between BCG-specific T cell responses and risk of disease, whereas more cytotoxic $CD4^+$ and $CD8^+$ T cell activity was associated with risk of tuberculosis. This lack of correlation between T cell responses and protection against tuberculosis demonstrates that immunity to tuberculosis is complex, and studies establishing the correlates of risk versus protection in tuberculosis would be useful in assessing the efficacy of the vaccine now available[23].

In the developing world, pregnant women often suffer chronic parasitic infections (such as malaria, filariasis, onchocerciasis and trypanosomiasis) that also infect the fetus via placental transfer of parasites or their soluble products. Christopher King (Cleveland, Ohio, USA) reported that such exposure stimulates an immune response in the fetus that can be strongly immunomodulatory and persist into childhood. Moreover, *in utero* exposure to helminth products during gestation diminishes the efficacy of vaccines such as BCG or the vaccine for Hib in infants by dampening the amount of IFN-γ or antigen-specific IgG, respectively. The effects of fetal exposure to parasites or their products on the development of immune responses in the face of multiple coinfections, and the degree to which pathogen-specific immune responses acquired *in utero* persist into early life, remain poorly understood.

Models, tools and methods

Animals can be used to obtain critical knowledge about human infant immune responses. They allow carefully controlled studies, the application of *in vivo* imaging technologies, the ready availability of tissues for more in-depth assessment of immune parameters, and the use of genetic manipulation for mechanistic studies. Indeed, animal studies, particularly in the mouse, led the way to the discovery of the diminished T_H1 responses and 'preferen-

Box 1 Areas of infant immunity that require further study

- Molecular mechanisms that regulate innate and adaptive immune responses
- Role of newly generated lymphocyte populations in immune responses
- Effect of epigenetics on the development of the infant immune system
- Mucosal immune development
- Regulatory T cells: development and mechanisms of suppression
- Immune memory development and persistence throughout life
- Early diagnosis of infants with PID
- Contributing factors in childhood asthma
- Nutrition, seasonal influences, severe infections and immune development
- Maternal antibodies and mechanisms of suppression
- Vaccine interference
- Therapeutic efficacy and mechanism of action of IVIg
- Re-evaluation of vaccine schedules
- Improvement of vaccine efficacy with adjuvants
- Identification of correlates of protective immunity
- Infant immunity in chronic infection or in coinfection
- Coupling of *in vivo* animal studies with *in vitro* human studies
- Application of global systems-based approaches

tial' T_H2 responses of infants[10]. Those studies demonstrated that the infant immune system is able to respond to infection, albeit in a qualitatively different manner than that of an adult. In addition, infant mice or larger animal models have been very beneficial in evaluating the efficacy of various adjuvants in activating the innate immune response[6]. Such studies have been instrumental in demonstrating that the infant immune system is able to develop fully mature inflammatory responses under the appropriate conditions of stimulation. Animal studies are also important for increasing the understanding of mechanisms underlying the unique responses of infants. Studies evaluating responses to pneumococcal vaccine in mice, for example, have shown that dampened responses to TLR agonists and lower expression of major histocompatibility complex class II in macrophages may contribute to greater susceptibility to pneumococcal colonization[24]. Clearly, much knowledge has been gained from animal studies; nonetheless, it is important to directly test the effects of candidate adjuvants identified in animals on cells from the human infant immune system. It would probably be most beneficial to couple *in vivo* studies in animals with *in vitro* models of human vaccine responses to evaluate safety, efficacy and vaccine interactions as well as to identify appropriate correlates of safety and protection or biomarkers during the course of disease or treatment.

Another challenge in the study of infant immunity is the availability of samples. Assays that can be miniaturized and that will provide the appropriate assessment of immune responses will greatly improve the ability to gain important information about the immune system of infants. As an example, Octavio Ramilo (Columbus, Ohio, USA) demonstrated an alternative method that can be used for diagnosis: transcriptional analysis of whole blood from infants to distinguish between viral and bacterial gene-expression signatures. Such an array analysis may be used to study host responses to pathogens and may permit better characterization of disease severity and disease process. Additionally, conclusions based solely on summary statistics may be inadequate, as they may mask critical information. Outliers in a sample set may deserve further analysis. In addition, systems-based approaches that include microarrays or deep sequencing, microRNA analysis, polychromatic flow cytometry, genomics and proteomics can be used and standardized so data obtained from different sample sets from the same site or different sites can be compared. Mechanistic studies using small interfering RNA and distinct neonatal plasma factors such as maternal antibodies, complement, adenosine and others can be studied for their effect on the infant's immune response. Elucidating the immune mechanisms and pathways involved in disease pathogenesis and identifying biomarkers to assess disease progression in infants will greatly aid in disease management.

Conclusion

This workshop was held with the ultimate goal of understanding the immune status of infants and improving the immune health of infants worldwide. Leaders in the field were brought together to discuss the present base of scientific knowledge on infant immunity (**Fig. 2**); to identify research gaps in understanding immune mechanisms in the infant that affect the development of protective immunity to infections; and to foster collaborations. The workshop brought to light areas in which further studies are needed in the field of infant immunity (**Box 1**). These include characterizing the innate and adaptive immune responses in general, improving vaccine regimens for resource-sufficient and resource-limited countries, improving detection methods for identifying PID, identifying new adjuvants and other immune-based therapies, and developing tools and appropriate model systems. The NIAID is committed to supporting studies that explore immune mechanisms in the infant that affect the response to infections and vaccines, nurturing discoveries in the earliest stages to push them to greater maturity, and identifying promising research and facilitating its translation into vaccines and treatments to improve the health of infants in the United States and around the world.

COMPETING FINANCIAL INTERESTS

The authors declare competing financial interests: details accompany the full-text HTML version of the paper at http://www.nature.com/natureimmunology/.

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Erratum: Challenges in infant immunity: implications for responses to infection and vaccines

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In the version of this article initially published, the length of term gestation in the fourth sentence is incorrect. This should read "(after 37 weeks of gestation)." The error has been corrected in the HTML and PDF versions of the article.