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"EDUCATING" THE NEONATAL IMMUNE SYSTEM: IMPLICATIONS FOR MUCOSAL IMMUNIZATION EARLY IN LIFE

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SUMMARY

New-borns and infants under six months of age are highly susceptible to infectious diseases. Vaccines that can safely and effectively prevent life-threatening illnesses during the first year of life are sorely needed. Several challenges remain for successful early-life immunization, the two most important being: 1) understanding the structure of the neonatal immune system and the mechanisms underlying neonatal responses; and 2) identifying vaccine strategies that can efficiently engage the fully competent, yet "inexperienced," neonatal immune system. Vaccines and adjuvants that can stimulate innate immune defences, enhance the maturation of dendritic cells and promote Th1-type signals have shown great promise in animal models and humans. An ideal vaccine for this age group would be capable of inducing long-lasting systemic and mucosal immunity bypassing maternal antibodies and would require minimal dosing via a user-friendly route. Vaccines that can be administered mucosally hold great promise because they can mount an immune response at the site of infection and would be practical for large-scale immunization. Identifying the requirements for "educating" the infant immune system will be essential for developing safe and effective vaccines for paediatric immunization.

INTRODUCTION

New-borns and very young infants are highly susceptible to infectious organisms, which cause high rates of morbidity and mortality (*Siegrist*, 2007; *Wilson* and *Kollmann*, 2008). Safe and effective immunization early in life could significantly reduce this burden, but the development of vaccines for the very young has been beset by many challenges. New-borns do not respond to T cell-independent polysaccharide vaccines (*Mond* and *Kokai-Kun*, 2008) and mount modest and short-lived antibody responses to T cell-dependent antigens, which require booster immunizations up to the second year of life (*Adkins* et al., 2004; *Siegrist*, 2007). The Th2-type environment that remains after the gestation period makes it difficult to induce Th1-type cell-mediated immunity (*Wegmann* et al., 1993). Maternal antibodies, although helpful for preventing infections during the first months of life, interfere with vac-

cine take and even residual levels can abrogate responses to routine vaccines such as measles, polio, rotavirus, tetanus, diphtheria, pertussis and *Haemophilus influenzae* type b (Hib). This creates a window of vulnerability against these pathogens that spans up to nine months of age (*Siegrist* et al., 1998).

There has been significant progress in recent years in our understanding of the functional competency of the neonatal/infant immune system. Neonatal dendritic cells (DC) and the capacity of these cells for antigen presentation and cytokine production are at the centre of the debate (*Ridge* et al., 1996). Both in animals and humans, neonatal DC exhibit an "immature" phenotype, with low levels of expression of MHC class II and co-stimulatory molecules and a limited capability for production of IL-12 and pro-inflammatory cytokines. As a result, neonatal DC have a reduced capacity to present foreign antigens and to stimulate naïve T cells compared with their adult DC counterparts (*Gans* et al., 1999). In contrast, neonatal T cells are able to undergo expansion and differentiation into antigen-specific effector and memory cells, but they require fully functional DC and cytokine signals. Neonatal B cell responses are also compromised due to the suboptimal DC function. The inefficient stimulation of T cells limits $CD4^+$ T cell help, which impairs the normal processes of B cell stimulation, migration to the germinal centres, avidity maturation, isotype class switching, and differentiation into either long-lived plasma or memory B cells. As a consequence, the B cell responses are usually feeble, transient and markedly inefficient.

There is substantial evidence suggesting that the limitations discussed above can be overcome and that neonatal immune responses can be induced when vaccine antigens are administered in the appropriate immunologic context. Hence, current research efforts are aimed at identifying vaccines and adjuvants that are capable of providing immunostimulatory signals that would safely and efficiently engage the "inexperienced," but fully competent, neonatal immune system.

NEW-BORNS AND INFANTS RESPOND EFFICIENTLY TO MICROBIAL ANTIGENS

Immune responses to a variety of microbial antigens can develop *in utero* after maternal infection. Functionally mature $CD8^+$ T cell responses were found in new-borns exposed to congenital infection with cytomegalovirus (CMV) (*Marchant* et al., 2003; *Gibson* et al., 2004) and *Trypanosoma cruzii* (*Hermann* et al., 2002). Immune responses that originated *in utero* after maternal immunization have also been reported; new-borns from mothers vaccinated against influenza during pregnancy developed virus-specific IgM antibodies and $CD4^+$ T cells that were present at birth (*Rastogi* et al., 2007).

Similarly, new-borns and young infants can mount potent adaptive immunity when exposed to pathogens soon after birth. Cytotoxic $CD8^+$ T cell responses were reported in infants infected with respiratory syncytial virus (RSV) and human immunodeficiency virus (HIV) (*Collins* et al., 1990). Cytokine-secreting $CD4^+$ T cells were also found in infants infected with herpes simplex virus (HSV) at birth. In many instances, these responses were comparable in magnitude and quality to those found in adults (*Hermann* et al., 2002).

Live attenuated vaccines have proven to be highly immunogenic when they are administered during the neonatal period. The classical example is the bacillus Calmette-Guerin (BCG), which for decades has been given to new-borns at birth and is still widely used in the developing world (*Andersen* and *Doherty*, 2005). Immunization with BCG at birth or at two months of age elicits a $CD4^+$ Th1-type response to *M. tuberculosis* antigens that is similar to the response in adults (*Ota* et al., 2002; *Marchant* et al., 1999). The diphtheria-tetanus whole cell pertussis (DTwP) vaccine induces Th1-type responses in one- to four-month-old infants that are comparable with those induced by *B. pertussis* infection (*Mascart* et al., 2003). Oral polio vaccine has been given to human new-borns and was found to elicit intestinal and serum antibody responses (*Halsey* and *Galazka*, 1985).

In contrast to the responses induced by live organisms, subunit vaccines such as hepatitis B, diphtheria-tetanus toxoid and Hib-tetanus toxoid conjugates primarily elicit antibodies and Th2-type cytokine-secreting $CD4^+$ T cells in young infants. These responses are usually undetectable after the first dose, necessitating several doses to generate sustained immunity. Hepatitis B surface antigen (HBSAg) is the only vaccine given at birth in industrialized countries and represents the best example that neonatal vaccination is feasible and effective (*Delage* et al., 1993). In response to HBSAg, infants develop antibody levels above those of adults, but very poor cellular responses (*Ota* et al., 2004). Likewise, the diphtheria-tetanus-acellular pertussis (DTaP) vaccine elicits Th2-type responses (*Rowe* et al., 2001). Because they are limited in their ability to elicit robust cell-mediated immunity, subunit vaccines fail to adequately protect new-borns against intracellular organisms, and the Th2-biased responses increase the risk for allergy and other undesirable immunologic responses.

Collectively, these examples support the argument that there are no insurmountable intrinsic defects in the neonatal immune system that would prevent successful immunization and that new-borns have the capacity to respond to properly designed vaccines.

VACCINES CAN INDUCE POTENT ADAPTIVE IMMUNITY DURING THE NEONATAL PERIOD

Extensive studies in animal models have shown that new-borns can develop Th1-type immunity, including adult-like $CD8^+$ cytotoxic lymphocytes (CTL) in response to live replicating viruses (*Sarzotti* et al., 1996; *VanCott* et al., 2006), bacteria (*Eisenberg* et al., 2003; *Roduit* et al., 2002; *Rayevskaya* et al., 2002) and DNA vaccines (*Hassett* et al., 2000; *Zhang* et al., 2002; *Capozzo* et al., 2006). Vaccine antigens can elicit potent immune responses during the neonatal period if they are accompanied by immunomodulatory molecules and adjuvants that activate innate immunity and enhance DC maturation and function, for example LPS (*Dadaglio* et al., 2002; *Ismaili* et al., 2003), CpG oligonucleotides (*Kovarik* et al., 1999), Flt 3 (*Vollstedt* et al., 2003), polyriboinosinic:polyribocytidilic acid (*Ma* and *Ross*, 2005) and cytokines, including IL-12 (*Pertmer* et al., 2001; *Sabirov* and *Metzger*, 2006), GM-CSF (*Capozzo* et al., 2003) and IFN-γ (*Pertmer* et al., 2001).

Activation of neonatal DC is regarded as the "key" step to induce

adaptive immunity at early stages of life. Functionally mature neonatal DC can efficiently present vaccine antigens and stimulate naïve $CD4^+$ T cells. They can also trigger a cascade of cytokines that will further enhance and sustain the resulting response. In the presence of mature DC, both mouse and human neonatal T cell function increases to adult levels (*Adkins* et al., 2004). Interestingly, although neonatal human DC may have a limited capacity to present antigens to $CD4^+$ T cells through the class II pathway (*Canaday* et al., 2006), they are fully competent to process and present antigens to $CD8^+$ T cells through the MHC class I antigen processing pathway (*Gold* et al., 2007).

Our group has shown that new-born mice immunized intranasally with *S. typhi* or *S. typhimurium* carrying tetanus toxoid Fragment C developed adult-like Fragment C antibody responses, mucosal antibody-secreting cells and T cell responses even in the presence of maternal antibodies (*Capozzo* et al., 2006). Vigorous priming of the neonatal immune system was demonstrated in new-borns immunized intranasally with *S. typhi* expressing *Yersinia pestis* F1 antigen, and this priming was associated with the capacity of Salmonella to enhance DC maturation (*Ramirez* et al., 2009). The signalling of bacterial components (for example, LPS, OMP and CpG motifs) through toll-like receptors (TLR) facilitates DC recruitment, maturation and migration to secondary lymph nodes and synthesis of IL-12 and Type 1 IFN (*Salazar-Gonzalez* and *McSorley*, 2005). These mature and activated DC stimulate the production of IFN-γ and IL-2 by $CD4^+$ T cells, promoting Th1-type responses. Activated $CD4^+$ T-helper cells expressing CD40L interact more efficiently with B cells, supporting Ig isotype switching, affinity maturation and immunologic memory.

A well-configured vaccine for neonatal immunization would be one that could activate innate immunity, contributing immunostimulatory (danger-like) signals that would enhance DC function for proper activation of neonatal T cells.

VACCINES THAT ACTIVATE INNATE IMMUNITY AND ENHANCE DC FUNCTION CAN SUCCESSFULLY STIMULATE THE IMMUNE SYSTEM IN EARLY LIFE

The neonatal immune system has remarkable plasticity and a capacity to mount potent adaptive immunity when appropriately stimulated. Vaccines and adjuvants that engage TLRs, activate neonatal DC and evoke Th1-type cytokines seem to be promising tools for successful neonatal immunization. These vaccines can "educate" the neonatal immune system by allowing neonatal DC to become fully functional antigen presenting cells that will subsequently stimulate T and B cells. An additional advantage of Th1-type vaccines is their potential capacity to prevent exacerbated Th2-type responses, which have been associated with allergic reactions. The exposure to foreign/environmental antigens and the higher incidence of natural infections with a variety of organisms early in life have been linked with a lower prevalence of allergy. This has been the theory supported by the "hygiene hypothesis" (*Schröder*, 2009).

Conceivably, stimulatory signals can imprint a state of "activation" on neonatal DC that could lead to improved responses against unrelated antigens given at the same time or even

later in life. Human infants that received BCG together with the HepB vaccine soon after birth had increased antibody and $CD4^+$ T cell responses to the HBSAg (Ota et al., 2002), which was thought to be associated with the capacity of BCG to activate neonatal DC. We have shown that mucosal priming of new-born mice with *S. typhi* can enhance responses to a recombinant subunit protein given by the parenteral route at a later time point (*Ramirez* et al., 2009). Ty21a, the only licensed oral typhoid vaccine, was safe and well tolerated when given to toddlers (< 24 months old), even at high doses ($1x10^9$ CFU), and could conceivably be used, like BCG, in younger children (*Murphy* et al., 1991). A new generation of rationally attenuated strains harbouring stabilized plasmids and non-antibiotic selection markers are being pursued as safer live-vector vaccine alternatives for the paediatric population. Ghost-particles derived from Gram-positive and Gram-negative organisms have emerged as promising candidates that are safe while offering the immunostimulatory properties of a living organism. These particles can deliver foreign antigens and are amenable for mucosal immunization. We have shown that ghost particles from *L. lactis* displaying *Y. pestis* LcrV elicit potent mucosal and systemic immunity and protect neonatally immunized mice from lethal systemic plague infection (*Ramirez* et al., 2009).

Vaccines that can be given to infants through mucosal routes (i.e., orally or intranasally) are of special interest not only because of the ease of administration, but also to reduce the interference of maternal immunity because lower concentrations of maternal antibodies are present in the infant mucosa compared with the circulation and systemic lymphoid tissues (*Siegrist*, 2003).

IMMUNIZATION REGIMENS THAT CAN ENHANCE VACCINE-INDUCED PROTECTIVE IMMUNITY EARLY IN LIFE

In neonatal animal models, the route of immunization can make a significant difference in the capacity to induce protective immunity (*Sabirov* and *Metzger*, 2006). We argue that immune responses to vaccines could be enhanced by using more efficient immunization regimes, such as a heterologous prime-boost approach. An advantage of a two-step immune stimulation is that the final response will include distinct features of both the initial prime and the subsequent boost. Particularly attractive are prime-boost strategies that combine mucosal and parenteral immunization because they extend the breath of the responses. A sindbis-based measles DNA vaccine primed 1-2-month-old infant rhesus macaques to develop a vigorous neutralizing antibody response to a subsequent boost with an aerosolized live attenuated measles vaccine (*Pasetti* et al., 2007). New-born mice primed mucosally with *S. typhi* expressing *Y. pestis* F1 and boosted parenterally with F1-alum elicited a high-avidity F1-specific IgG response, mucosal antibody-secreting cells and T cell responses that surpassed those elicited by repeated immunization with *S. typhi* (F1) or F1-alum (*Ramirez* et al., 2009). A prime-boost strategy could also allow tailoring of the immune response to favour the responses necessary for protection. An obvious drawback of the prime-boost approach, however, is the requirement of more than one vaccina-

tion encounter, in which case priming in the form of an easily administered mucosal vaccine would advantageous. The success of prime-boost immunization in human new-borns and infants remains to be determined.

EARLY-LIFE IMMUNIZATION, TOLERANCE AND AUTOIMMUNITY: SHOULD WE BE CONCERNED?

A valid question related to neonatal vaccination is whether the introduction of foreign antigens early in life could induce tolerance or predispose recipients for hyporesponsiveness later in life. There is limited evidence indicating that this may actually occur in humans [Reviewed in (*Siegrist*, 2001)]. The manner, the context and the route by which antigens are introduced to the immune system are critical in determining whether an immune response or a lack of thereof will ensue. We argue that the development of tolerance is less likely when antigens are administered with a potent immune stimulation, in which case the Th1-type vaccines and adjuvants could lessen such a risk. Antigens delivered in particulate as opposed to soluble form are also less likely to induce tolerance. Reduced responses to DTaP have been reported when new-borns received a dose at birth followed by routine immunization at 2, 4, 6 and 17 months (*Halasa* et al., 2008). It has been noted, however, that this type of hyporesponsiveness likely reflects vaccine interference rather than neonatal immunological impairment (*Siegrist*, 2008) because a number of studies actually described enhanced vaccine effectiveness in new-borns immunized with acellular pertussis (aP) at birth prior to the routine DTaP immunization (*Knuf* et al., 2008; *Belloni* et al., 2003).

It has also been questioned whether neonatal vaccination could abrogate self-tolerance and lead to autoimmune diseases. Millions of new-borns have been vaccinated at birth with BCG, polio and HepB vaccines, and there is no evidence of an increased incidence of autoimmunity associated with perinatal immunization (*Belloni* et al., 2002). The mechanisms that induce tolerance to self-antigens (both systemically and mucosally) are not restricted to early life but are active throughout life. Thus, it can be argued that the neonatal period is not at higher risk for autoimmunity than any other stage in life. Regulatory T cells have a central role in the maintenance of tolerance. $CD4^{+}CD25^{+}$ Treg cells have been found in cord-blood (*Takahata* et al., 2004) and likely have a key role in controlling potentially harmful responses.

"EDUCATING" THE EARLY-LIFE IMMUNE SYSTEM TO OVERCOME THE TOLEROGENIC BARRIER FOR ORAL IMMUNIZATION

The "education" of the neonatal/infant immune system in the mucosal compartment has unique features that complement the education of immune cells in the systemic compartment. A state of hyporesponsiveness (which increases with age) prevails in the mucosal lymphoid tissue, particularly in the gastrointestinal tract, due to the massive and continuous exposure to foreign antigens. This tissue, however, can adapt to maintain a delicate balance between the

need to maintain immunologic silence or to respond to potentially harmful agents. Human milk promotes the development and maturation of the mucosal-associated lymphoid tissue and contains numerous immunomodulatory components (such as TGF-β and Vitamin A) that influence immunological competency. Exposure to food and other (non-self) antigens in breast milk favours the development of tolerance in rodents (*Verhasselt*, 2010). Infant breast-feeding has been associated with a reduced incidence of allergy and asthma, which could be explained by the presence of maternal antibodies that prevent respiratory viral infections, but also by the tolerogenic presentation of allergens [Reviewed in (*Verhasselt*, 2010)]. Interestingly, the commensal flora in the gut also enhances the development, maturation and functional capacity of immune cells in the gut (*Eberl* and *Lochner*, 2009). It is therefore conceivable that the processes that down-regulate immune responses to prevent disease in the intestinal environment may also interfere with vaccine take.

A number of routine (e.g., polio, rotavirus) and experimental (e.g., Shigella, cholera) vaccines have performed sub-optimally in developing countries compared with industrialized ones [Reviewed in (*Walker* et al., 2005, 2007; *Mirzayeva* et al., 2009)]. Several reasons have been proposed to explain this observation, including malnutrition of infants and mothers, micronutrient deficiencies, parasite and bacterial co-infections and maternal antibodies in breast milk (*Czerkinsky* and *Holmgren*, 2009). It has also been proposed that infants in developing countries (and their mothers) have a more diverse and frequent exposure to microbial antigens, and as a result, they have a more mature (and tolerogenic) immune system (*Czerkinsky* and *Holmgren*, 2009). The lower prevalence of allergy in the developing world compared with industrialized regions is also well known. If the "tolerogenic environment" of the gastrointestinal tract is indeed a factor contributing to the lower immune responses to enteric vaccines, administering these vaccines earlier (possibly at birth) could conceivably circumvent this limitation. Well-tolerated and effective mucosal adjuvants that could activate innate immune cells and trigger an adequate level of pro-inflammatory signals could also break this "tolerogenic" barrier. This concept is worth further study in animal models and *in vitro*, possibly using engineered 3D tissue-culture model systems.

CONCLUSION

Vaccination during the neonatal period could dramatically reduce disease burden and risk of infection during the first year of life. Since health care is sought at birth, perinatal vaccination can have a further outreach in areas where they are most needed. A challenge that remains is identifying vaccines and adjuvants that can provide immune stimulatory "danger" signals to efficiently activate the inexperienced neonatal immune system, without compromising safety. Neonatal immune cells have great plasticity and the potential to be "educated." Vaccines that hold great promise are those that stimulate innate immunity, promote activation and maturation of neonatal DC and allow for intracellular antigen delivery (to shield vaccine antigens from maternal antibodies). Ideally, such a vaccine could be given at birth,

through a mucosal route, and would be effective after a single dose. Neonatal immunization seems to pose a low risk for autoimmune disease and tolerance, but reactogenicity must be closely examined to achieve the appropriate risk/benefit balance. Early-life immunization and the use of well-tolerated and effective mucosal adjuvants could help overcome the tolerogenic barrier for enteric vaccine delivery.

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LITERATURE

Adkins, B., Leclerc, C., and Marshall-Clarke, S.: Neonatal Adaptive immunity comes of age. Nat. Rev. Immunol. 4, 553-564 (2004).

Andersen, P. and Doherty, T.M.: The success and failure of BCG - Implications for a novel tuberculosis vaccine. Nat. Rev. Microbiol. 3, 656-662 (2005).

Belloni, C., Avanzini, M.A., De, S.A., Martinetti, M., Pasi, A., Coslovich, E., Autelli, M., Masanti, M.L., Cuccia, M., Tinelli, C., Rondini, G., and Lorini, R.: No evidence of autoimmunity in 6-year-old children immunized at birth with recombinant hepatitis B vaccine. Pediatrics 110, e4 (2002).

Belloni, C., De, S.A., Tinelli, C., Avanzini, M.A., Marconi, M., Strano, F., Rondini, G., and Chirico, G.: Immunogenicity of a three-component acellular pertussis vaccine administered at birth. Pediatrics 111, 1042-1045 (2003).

Canaday, D.H., Chakravarti, S., Srivastava, T., Tisch, D.J., Cheruvu, V.K., Smialek, J., Harding, C.V., and Ramachandra, L.: Class II MHC antigen presentation defect in neonatal monocytes is not correlated with decreased MHC-II expression. Cell. Immunol. 243, 96-106 (2006).

Capozzo, A.V., Pistone, C.V., Dran, G., Fernandez, G., Gomez, S., Bentancor, L.V., Rubel, C., Ibarra, C., Isturiz, M., and Palermo, M.S. Development of DNA vaccines against hemolytic-uremic syndrome in a murine model. Infect. Immun. 71, 3971-3978 (2003).

Capozzo, A.V., Ramirez, K., Polo, J.M., Ulmer, J., Barry, E.M., Levine, M.M., and Pasetti, M.F.: Neonatal immunization with a sindbis virus-DNA measles vaccine induces adult-like neutralizing antibodies and cell-mediated immunity in the presence of maternal antibodies. J. Immunol. 176, 5671-5681 (2006).

Collins, W.E., Nussenzweig, R.S., Ruebush, T., Bathurst, I.C., Nardin, E.H., Gibson, H.L., Campbell, G.H., Barr, P.J., Broderson, J.R., Skinner, J.C., Filipski, V.K., Stanfill, P.S., Roberts, J.M., AND Carla L. Wilson, C.L.: Further studies on the immunization of Saimiri sciureus boliviensis with recombinant vaccines based on the circumsporozoite protein of Plasmodium vivax. Am. J. Trop. Med. Hyg. 43, 576-583 (1990).

Czerkinsky, C. and Holmgren, J.: Enteric vaccines for the developing world: A challenge for mucosal immunology. Mucosal Immunol. 2, 284-287 (2009).

Dadaglio, G., Sun, C.M., Lo-Man, R., Siegrist, C.A., and Leclerc, C.: Efficient in vivo priming of specific cytotoxic T cell responses by neonatal dendritic cells. J. Immunol. 168,.2219-2224 (2002).

Delage, G., Remy-Prince, S., and Montplaisir, S.: Combined active-passive immunization against the hepatitis B virus: Five-year fol-

low-up of children born to hepatitis B surface antigen-positive mothers. Pediatr. Infect. Dis. J. 12, 126-130 (1993).

Eberl, G. and Lochner, M.: The development of intestinal lymphoid tissues at the interface of self and microbiota. Mucosal Immunol. 2, 478-485 (2009).

Eisenberg, J.C., Czinn, S.J., Garhart, C.A., Redline, R.W., Bartholomae, W.C., Gottwein, J.M., Nedrud, J.G., Emancipator, S.E., Boehm, B.B., Lehmann, P.V., and Blanchard, T.G.: Protective efficacy of anti-Helicobacter pylori immunity following systemic immunization of neonatal mice. Infect. Immun. 71, 1820-1827 (2003).

Gans, H.A., Maldonado, Y., Yasukawa, L.L., Beeler, J., Audet, S., Rinki, M.M., DeHovitz, R., and Arvin, A.M.: IL-12, IFN-gamma, and T cell proliferation to measles in immunized infants. J. Immunol. 162, 5569-5575 (1999).

Gibson, L., Piccinini, G., Lilleri, D., Revello, M.G., Wang, Z., Markel, S., Diamond, D.J., and Luzuriaga, K.: Human cytomegalovirus proteins Pp65 and immediate early protein 1 are common targets for CD8+ T cell responses in children with congenital or postnatal human cytomegalovirus infection. J. Immunol. 172, 2256-2264 (2004).

Gold, M.C., Robinson, T.L., Cook, M.S., Byrd, L.K., Ehlinger, H.D., Lewinsohn, D.M., and Lewinsohn, D.A.: Human neonatal dendritic cells are competent in MHC class I antigen processing and presentation. PLoS ONE 2, e957 (2007).

Halasa, N.B., O'Shea, A., Shi, J.R., Lafleur, B.J. and Edwards, K.M.: Poor immune responses to a birth dose of diphtheria, tetanus, and acellular pertussis Vaccine J. Pediatr. 153, 327-332 (2008).

Halsey, N. and Galazka, A.: The efficacy of DPT and oral poliomyelitis immunization schedules initiated from birth to 12 weeks of age. Bull. World Health Organ. 63, 1151-1169 (1985).

Hassett, D.E., Zhang, J., Slifka, M., and Whitton, J.L.: Immune responses following neonatal DNA vaccination are long-lived, abundant, and qualitatively similar to those induced by conventional immunization. J. Virol. 74, 2620-2627 (2000).

Hermann, E., Truyens, C., Alonso-Vega, C., Even, J., Rodriguez, P., Berthe, A., Gonzalez-Merino, E., Torrico, F., and Carlier, Y.: Human fetuses are able to mount an adultlike CD8 T-cell response. Blood 100, 2153-2158 (2002).

Ismaili J, van der Sande, M., Holland, M.J., Sambou, I., Keita, S., Allsopp, C., Ota, M.O., McAdam, K.P., and Pinder, M.: Plasmodium falciparum infection of the placenta affects newborn immune responses. Clin. Exp. Immunol. 133, 414-421 (2003).

Knuf, M., Schmitt, H.J., Wolter, J., Schuerman, L., Jacquet, J.M., Kieninger, D., Siegrist, C.A., and Zepp, F.: Neonatal vaccination with an acellular pertussis vaccine accelerates the acquisition of pertussis antibodies in infants. J. Pediatr. 152, 655-660 (2008).

Kovarik, J., Bozzotti, P., Love-Homan, L., Pihlgren, M., Davis, H.L., Lambert, P.H., Krieg, A.M., and Siegrist, C.A.: CpG oligodeoxynucleotides can circumvent the Th2 polarization of neonatal responses to vaccines but may fail to fully redirect Th2 responses established by neonatal priming. J. Immunol. 162, 1611-1617 (1999).

Ma, Y. and Ross, A.C.: The anti-tetanus immune response of neonatal mice is augmented by retinoic acid combined with polyriboinosinic:polyribocytidylic acid. Proc. Natl. Acad. Sci. USA 102, 13556-13561 (2005).

Marchant, A., Appay, V., van der Sande, M., Dulphy, N., Liesnard, C., Kidd, M., Kaye, S., Ojuola, O., Gillespie, G.M., Vargas Cuero, A.L., Cerundolo, V., Callan, M., McAdam, K.P., Rowland-Jones, S.L., Donner, C., McMichael, A.J., and Whittle, H.: Mature CD8(+) T lymphocyte response to viral infection during fetal life. J. Clin. Invest. 111, 1747-1755 (2003).

Marchant, A., Goetghebuer, T., Ota, M.O., Wolfe, I., Ceesay, S.J., De Groote, D., Corrah, T., Bennett, S., Wheeler, J., Huygen, K., Aaby, P., McAdam, K.P., and

Newport, M.J.: Newborns develop a Th1-Type immune response to Mycobacterium bovis bacillus Calmette-Guerin vaccination. J. Immunol. 163, 2249-2255 (1999).

Mascart, F., Verscheure, V., Malfroot, A., Hainaut, M., Pierard, D., Temerman, S., Peltier, A., Debrie, A.S., Levy, J., Del, G.G., and Locht, C.: Bordetella pertussis infection in 2-month-old infants promotes type 1 T cell responses. J. Immunol. 170, 1504-1509 (2003).

Mirzayeva, R., Steele, A.D., Parashar, U.D., Zaman, K., Neuzil, K.M., and Nelson, E.A.: Evaluation of rotavirus vaccines in Asia--Are there lessons to be learnt? Vaccine 27 Suppl 5, F120-F129 (2009).

Mond, J.J. and Kokai-Kun, J.F.: The multifunctional role of antibodies in the protective response to bacterial T cell-independent antigens. Curr. Top. Microbiol. Immunol. 319, 17-40 (2008).

Murphy, J.R., Grez, L., Schlesinger, L., Ferreccio, C., Baqar, S., Munoz, C., Wasserman, S.S., Losonsky, G., Olson, J.G., and Levine, M.M.: Immunogenicity of Salmonella typhi Ty21a vaccine for young children. Infect. Immun. 59, 4291-4293 (1991).

Ota, M.O., Vekemans, J., Schlegel-Haueter, S.E., Fielding, K., Sanneh, M., Kidd, M., Newport, M.J., Aaby, P., Whittle, H., Lambert, P.H., McAdam, K.P., Siegrist, C.A., and Marchant, A.: Influence of Mycobacterium bovis bacillus Calmette-Guerin on antibody and cytokine responses to human neonatal vaccination. J. Immunol. 168, 919-925 (2002).

Ota, M.O., Vekemans, J., Schlegel-Haueter, S.E., Fielding, K., Whittle, H., Lambert, P.H., McAdam, K.P., Siegrist, C.A., and Marchant, A.: Hepatitis B immunisation induces higher antibody and memory Th2 responses in new-borns than in adults. Vaccine 22, 511-519 (2004).

Pasetti, M.F., Resendiz-Albor, A., Ramirez, K., Stout, R., Papania, M., Adams, R.J., Polack, F.P., Ward, B.J., Burt, D., Chabot, S., Ulmer, J., Barry, E.M., and Levine, M.M.: Heterologous prime-boost strategy to immunize very young infants against measles: Pre-clinical studies in rhesus macaques. Clin. Pharmacol. Ther. 82, 672-685 (2007).

Pertmer, T.M., Oran, A.E., Madorin, C.A., and Robinson, H.L.: Th1 genetic adjuvants modulate immune responses in neonates. Vaccine 19, 1764-1771 (2001).

Ramirez, K., Capozzo, A.V., Lloyd, S.A., Sztein, M.B., Nataro, J.P., and Pasetti, M.F.: Mucosally delivered Salmonella typhi expressing the Yersinia pestis F1 antigen elicits mucosal and systemic immunity early in life and primes the neonatal immune system for a vigorous anamnestic response to parenteral F1 boost. J. Immunol. 182, 1211-1222 (2009).

Rastogi, D., Wang, C., Mao, X., Lendor, C., Rothman, P.B., and Miller, R.L.: Antigen-specific immune responses to influenza vaccine in utero. J. Clin. Invest. 117, 1637-1646 (2007).

Rayevskaya, M., Kushnir, N., and Frankel, F.R.: Safety and immunogenicity in neonatal mice of a hyperattenuated Listeria vaccine directed against human immunodeficiency iirus. J. Virol. 76, 918-922 (2002).

Ridge, J.P., Fuchs, E.J., and Matzinger, P.: Neonatal tolerance revisited: Turning on newborn T cells with dendritic cells. Science 271, 1723-1726 (1996).

Roduit, C., Bozzotti, P., Mielcarek, N., Lambert, P.H., Del Giudice, G., Locht, C., and Siegrist, C.A.: Immunogenicity and protective efficacy of neonatal vaccination against Bordetella pertussis in a murine Model: Evidence for early control of Pertussis. Infect. Immun. 70, 3521-3528 (2002).

Rowe, J., Macaubas, C., Monger, T., Holt, B.J., Harvey, J., Poolman, J.T., Loh, R., Sly, P.D., and Holt, P.G.: Heterogeneity in diphtheria-tetanus-acellular pertussis vaccine-specific cellular immunity during infancy: Relationship to variations in the kinetics of postnatal maturation of systemic Th1 function. J. Infect. Dis. 184, 80-88 (2001).

Sabirov, A. and Metzger, D.W.: Intranasal vaccination of neonatal mice with polysaccharide conjugate vaccine for protection against pneumococcal otitis media. Vaccine 24, 5584-5592 (2006).

Salazar-Gonzalez, R.M. and McSorley, S.J.: Salmonella flagellin, a microbial target of the innate and adaptive immune system. Immunol. Lett. 101, 117-122 (2005).

Sarzotti, M., Robbins, D.S., and Hoffman, P.M.: Induction of protective CTL responses in newborn mice by a murine retrovirus. Science 271, 1726-1728 (1996).

Schröder, N.W.: The role of innate immunity in the pathogenesis of asthma. Curr. Opin. Allergy Clin. Immunol. 9, 38-43 (2009).

Siegrist, C.A.: Neonatal and early life vaccinology. Vaccine 19, 3331-3346 (2001).

Siegrist, C.A.: Mechanisms by which maternal antibodies influence infant vaccine responses: Review of hypotheses and definition of main determinants. Vaccine 21, 3406-3412 (2003).

Siegrist, C.A.: The challenges of vaccine responses in early life: Selected examples. J. Comp. Pathol. 137, Suppl 1, S4-S9 (2007).

Siegrist, C.A.: Blame vaccine interference, not neonatal immunization, for suboptimal responses after neonatal diphtheria, tetanus, and acellular Pertussis immunization. J. Pediatr. 153, 305-307 (2008).

Siegrist, C.A., Cordova, M., Brandt, C., Barrios, C., Berney, M., Tougne, C., Kovarik, J., and Lambert, P.H.: Determinants of infant responses to vaccines in presence of maternal antibodies. Vaccine 16, 1409-1414 (1998).

Takahata, Y., Nomura, A., Takada, H., Ohga, S., Furuno, K., Hikino, S., Nakayama, H., Sakaguchi, S., and Hara, T.: CD25+CD4+ T cells in human cord blood: An immunoregulatory subset with naive phenotype and specific expression of forkhead box P3 (Foxp3) gene. Exp. Hematol. 32, 622-629 (2004).

VanCott, J.L., Prada, A.E., McNeal, M.M., Stone, S.C., Basu, M., Huffer, B., Jr., Smiley, K.L., Shao, M., Bean, J.A., Clements, J.D., Choi, A.H., and Ward, R.L.: Mice develop effective but delayed protective immune responses when immunized as neonates either intranasally with nonliving VP6/LT(R192G) or orally with live rhesus rotavirus vaccine candidates. J. Virol. 80, 4949-4961 (2006).

Verhasselt, V.: Oral tolerance in neonates: From basics to potential prevention of allergic disease. Mucosal Immunol. 3, 326-333 (2010).

Vollstedt, S., Franchini, M., Hefti, H.P., Odermatt, B., O'Keeffe, M., Alber, G., Glanzmann, B., Riesen, M., Ackermann, M., and Suter, M.: Flt3 ligand-treated neonatal mice have increased innate immunity against intracellular pathogens and efficiently control virus Infections. J. Exp. Med. 197, 575-584 (2003).

Walker, R.I., Steele, D., and Aguado, T.: Analysis of strategies to successfully vaccinate infants in developing countries against enterotoxigenic E. coli (ETEC) disease. Vaccine 25, 2545-2566 (2007).

Walker, R.I., Van De Verg, L.L., Hall, R.H., Schmitt, C.K., Woo, K., and Hale, V.: Enteric vaccines for pediatric use. Workshop summary. Vaccine 23, 5432-5439 (2005).

Wegmann, T.G., Lin, H., Guilbert, L., and Mosmann, T.R.: Bidirectional cytokine interactions in the maternal-fetal relationship: Is successful pregnancy a TH2 phenomenon? Immunol. Today 14, 353-356 (1993).

Wilson, C.B. and Kollmann, T.R.: Induction of antigen-specific immunity in human neonates and infants. Nestle Nutr. Workshop Ser. Pediatr. Program 61, 183-195 (2008).

Zhang, J., Silvestri, N., Whitton, J.L., and Hassett, D.E.: Neonates mount robust and protective adult-like CD8(+)-T-cell responses to DNA vaccines. J. Virol. 76, 11911-11919 (2002).