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Heterologous ("Nonspecific") and Sex-Differential Effects of Vaccines: Epidemiology, Clinical Trials, and Emerging Immunologic Mechanisms

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A growing body of evidence from epidemiologic, clinical, and immunologic studies indicates that vaccines can influence morbidity and mortality independent of vaccine-specific B-cell or T-cell immunity. For example, the live attenuated measles vaccine and BCG vaccine may reduce mortality from infections other than measles or tuberculosis, respectively. Immunologists call these heterologous effects and epidemiologists have called them nonspecific effects, indicating that they manifest against a broad range of pathogens/disease. These effects differ by sex, can be beneficial or detrimental, and appear to be mediated by mechanisms including innate immune memory (also known as "trained immunity") and cross-reacting lymphocytes. Herein we review recent studies in this emerging field based on a meeting of experts, the recent Optimmunize meeting, held in Copenhagen, Denmark, in August 2012. Further characterization of these effects is likely to expand the way vaccines are evaluated and alter the manner and sequence in which they are given.

Keywords. heterologous effects; nonspecific effects; vaccine; sex-differential effects; innate immunity.

A growing number of randomized trials and observational studies suggest that some vaccines influence morbidity and mortality independent of vaccine-specific B-cell or T-cell immunity. Immunologists refer to these as heterologous effects and epidemiologists call them "nonspecific effects." Some of these heterologous effects are beneficial and thus might be exploited in vaccine schedules. Other vaccine heterologous effects may be detrimental, depending on the sex of the recipient and the timing and sequence of vaccination. A group of international experts involved in epidemiologic or laboratory studies relevant to vaccine heterologous effects formed a consortium called "Optimmunize" in 2010 [1]. A second meeting was held in August 2012 in Copenhagen, Denmark, to discuss the latest epidemiologic evidence and progress in elucidating the immunologic mechanisms for vaccine heterologous effects. Herein we review the exciting advances in this field.

A NEW PARADIGM IN VACCINOLOGY

The current paradigm holds that vaccines protect only against the target disease, with equivalent effects regardless of the order in which vaccines are given, the sex of the recipient, the season, or other variables. Accordingly, the major priority is securing 100% coverage of vaccine delivery. An alternative paradigm considers the immune system to be uniquely influenced by each vaccine given. In this model, vaccines have heterologous effects that influence the immune response to subsequent exposure to unrelated stimuli. These heterologous effects are influenced by the type of vaccine and the sequence of vaccination; they differ according

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to sex and when micronutrients are coadministered. It is important that heterologous effects are characterized in order that they might be exploited in future vaccine schedules and inform future vaccine design.

EPIDEMIOLOGIC EVIDENCE FOR HETEROLOGOUS EFFECTS OF VACCINES FROM RANDOMIZED TRIALS

Much vaccine development has been ad hoc, empiric, and focused mainly on adults [2]. Furthermore, vaccine trials almost exclusively investigate specific immune responses or the effect on the targeted disease, and not the overall effect on morbidity or mortality. However, data from 1932 showed that all-cause mortality among 20 000 children in the first 4 years of life was far lower in BCG-immunized infants than in non-BCG recipients [3]. Mortality from tuberculosis is not common in this age group and accounted for only a small fraction of the reduced deaths. However, there were dramatic reductions in neonatal deaths and deaths from nontuberculous causes in the BCG group. The author speculated that BCG induces "nonspecific immunity." Similar observations were made when smallpox vaccination was introduced 200 years ago [4].

Great care must be taken to adjust for potential confounders in observational studies. In the context of protective heterologous effects, the vaccinated groups may be those that are more likely to seek healthcare and thus survive. Randomized trials are therefore essential [5, 6]; indeed, the strongest evidence for the heterologous effects of vaccines comes from randomized studies of the BCG vaccine and measles vaccine (MV) in Guinea-Bissau. In one study, 2320 low-birth-weight infants, who are normally excluded from BCG vaccination at birth, were randomized to BCG or no BCG at birth. Those who received BCG had a 45% (95% confidence interval [CI], 11%–66%) decrease in mortality in the first 4 weeks of life [7]. Exposure to tuberculosis in the household was minimal; and standard verbal autopsy suggested that the reduced mortality was due to prevention of sepsis and respiratory infections, the commonest causes of death in low-birth-weight neonates in high-mortality settings [8].

Of note, different BCG strains elicit different immunologic responses, such as induced polyfunctional CD4 T cells and $CD107^{+}$ T cells, both believed to be important for protection against tuberculosis [9], and nonspecific cytokine responses to tetanus toxoid [10]. Two randomized trials led by Optimmunize members are presently ongoing in Melbourne, Australia, and Copenhagen, Denmark, in which almost 6000 newborn infants are being randomized to receive BCG or no BCG at birth. The primary endpoints of these studies are measures of allergy, respiratory tract infections, and admission to hospital with infection, as mortality is low in these settings. Innate and adaptive immunity in BCG-vaccinated and BCG-naive groups will also be studied to elucidate protective mechanisms. A randomized trial of the effect of giving BCG at birth to low-birth-weight babies is also planned in India.

Among children who had not received vitamin A at birth, a randomized trial in Guinea-Bissau demonstrated a 41% (95% CI, 11%–61%) decrease in all-cause mortality from 4.5 months to 36 months in those randomized to receive an additional MV at 4.5 months of age; only 6% of the 41% reduction was attributed to prevention of measles infection [11]. Indeed, other trials and community studies throughout Africa have similarly shown decreased mortality from causes other than measles among those receiving MV, especially among females [12]. Current World Health Organization (WHO) policy is for MV to be given at 9 months of age, as measles antibody seroconversion rates are inferior in infants younger than this age. Maternal antibodies may interfere with antibody induction by MV in infants, but recent studies in Guinea-Bissau suggest that the beneficial heterologous effects of MV are greater in infants who have maternal antibody at measles vaccination (P. Aaby, C. Martins, M. Garly, A. Andersen, A. B. Fisker, M. H. Claesson, H. Ravm, A. Rodrigues, H. C. Whittle, and C. S. Benn, submitted); the mechanism responsible for this is not known.

Worryingly, epidemiologic evidence suggests that some vaccines such as diphtheria, tetanus, and whole-cell pertussis vaccine (DTwP) may have harmful heterologous effects. Review of available observational studies and 2 randomized trials examining giving or not giving BCG or MV shortly after DTwP suggests that DTwP is associated with increased child mortality, especially in females [5, 13]. Indeed, increased female mortality following high-titer MV led to its withdrawal by WHO, although the increased mortality was subsequently found to have occurred only when DTwP was given after the high-titer MV [14]. The deleterious effects last for months after DTwP is given and are therefore quite distinct from the immediate endotoxin-mediated side effects of whole-cell pertussis vaccination [15].

Overall, studies evaluating the effects of vaccination on infant and child mortality suggest several tentative patterns: (1) potentially beneficial heterologous effects are most often observed with live vaccines such as BCG and MV, and also with oral polio vaccine [16] and smallpox vaccine [17]; (2) potentially detrimental heterologous effects have been noted with certain inactivated vaccines such as DTwP; (3) the order of vaccination can be important [13]; and (4) both the negative and positive heterologous effects appear to be stronger in female than in male infants.

VITAMIN A SUPPLEMENTATION HAS IMMUNOENHANCING EFFECTS INDEPENDENT OF VITAMIN A REPLETION

Randomized trials from the late 1980s/early 1990s demonstrated benefit of vitamin A supplementation given after 6 months of age [18]. Subsequently, WHO recommended that it be given every 4–6 months from 6 months to 5 years of age, and for logistic reasons administration was linked to vaccination contacts. The effect of combining vitamin A and vaccines had not been evaluated in randomized trials. Benn and colleagues have recently conducted the first randomized trial of vitamin A supplementation at vaccination in >7000 children after 6 months of age. Overall, vitamin A had no beneficial effect, but a strong sex-differential effect was observed with a approximately 35% reduction in mortality among females and a 70% increase in mortality in males in the next 12 months if vitamin A was given at the time of vaccination (A. B. Fisker, C. Bale, A. Rodrigues, I. Balde, M. Fernandes, M. J. Jørgensen, N. Danneskiold-Samsøe, L. Hornshøj, J. Rasmussen, E. D. Christensen, B. M. Bibby, P. Aaby, and C. S. Benn, submitted).

Studies of neonatal vitamin A supplementation (NNVAS) in Asia suggested decreased mortality [19–21], whereas studies in Africa suggest no benefit or increased mortality [22–25]. The overall conclusion from a systematic review is that NNVAS has no net effect on mortality, although this may be due to combining data from studies with opposing effects [26]. The effects on mortality appear to be unrelated to vitamin A status, suggesting they are mediated through distinct mechanisms. The effect seems to be beneficial as long as BCG is the most recent vaccine, but may become detrimental once children receive the inactivated DTwP vaccine [27]. Vitamin A has numerous effects on both innate and adaptive immunity, including modulation of helper T-cell 1 (Th1)/helper T-cell 2 (Th2) balance and differentiation of helper T-cell 17 (Th17) and regulatory T cells [28], any of which might be involved in the immune modulating effects of vitamin A. Overall, vitamin A appears to amplify both the specific (eg, measles antibody) and heterologous effects of vaccines in a sex-differential manner, even when the vaccines are given several months after the initial vitamin A supplementation [29, 30]. In light of the uncertainty and importance of this topic, WHO has commissioned 3 ongoing randomized controlled trials in Africa and Asia to study the effect of NNVAS on child mortality, and 3 detailed immunology studies to investigate the underlying mechanisms [31].

DISTINCT IMMUNITY OF NEWBORNS AND INFANTS IMPACTS VACCINE RESPONSES

The infant immune system is frequently described as "immature" and yet might be better considered as perfectly adapted to the different functional demands of early life [32]. Th1 cytokine production is suppressed in utero, probably reflecting the need to avoid allogenic reactions between the mother and fetus. In early life, newborns are rapidly exposed to multiple microbes and allergens to which they have no preexisting immunity, and overexuberant innate and adaptive inflammatory responses could be detrimental [33]. Human newborn plasma contains multiple soluble immune regulatory factors, including maternal antibodies, immunosuppressive interleukin 10 (IL-10), adenosine, prostaglandins, histamine, and others, all of which can limit neonatal proinflammatory/Th1-polarizing immune responses [34]. Term newborns demonstrate impaired production of Th1 polarizing cytokines with robust production of Th2- and Th17-polarizing and anti-inflammatory (eg, IL-10) cytokines [35]. Production of antiviral interferons gradually increases during the first weeks to months of life, paralleling the decline in susceptibility to viral pathogens.

Infant vaccine responses differ from those of adults, with age-dependent responses to vaccine adjuvants [36] and poor immunogenicity of certain vaccines (eg, polysaccharide vaccines) in early life [37]. Interestingly, BCG vaccine, attenuated *Mycobacterium bovis*, is self-adjuvanted via its ability to activate multiple Toll-like receptors (TLRs) and stimulates adult-level Th1 responses when administered to newborns [38]. A novel in vitro platform to assess vaccine responses has been developed, called the neonatal tissue construct (NTC). The NTC is a 3-dimensional microphysiologic system that enables generation of autonomously derived dendritic cells in the presence of immunomodulatory neonatal plasma [34]. Using this novel NTC platform, preliminary data indicate that BCG engenders a much stronger Th1 and autologous naive lymphocyte proliferative response than hepatitis B or pneumococcal conjugate vaccines [39]. Differential responses to vaccination early in life have also been studied using an in vivo newborn mouse model, demonstrating that certain TLR agonists enhance resistance of neonatal mice to subsequent bacterial infection [40]. Human neonatal dendritic cells are particularly responsive to agonists of TLR7/8 that are refractory to plasma adenosine inhibition and are candidate vaccine adjuvants [41]. Thus, innate immune stimulation via pattern recognition receptors (eg, TLRs) may provide one mechanism whereby susceptibility to heterologous infections is altered by vaccination. Indeed, taking adjuvant-mediated heterologous effects into consideration may ultimately improve the safety and efficacy of emerging pediatric vaccines.

CAN EPIGENETIC EFFECTS ON INNATE IMMUNITY ACCOUNT FOR VACCINE HETEROLOGOUS EFFECTS?

Early-life innate immune development appears to be driven by environmental challenges such as incidental exposure to

pathogens as well as vaccines [32, 42, 43]. Therefore, vaccines may not only induce adaptive immunity but also direct innate immune ontogeny. By standard immunologic theory, species that rely on innate immunity alone should have no immune memory; however, many manifest robust immune memory that in some cases can be transmitted epigenetically to the next generation [44]. Natural killer cells have memory characteristics and can self-renew and undergo multiple rounds of reexpansion [45, 46], and macrophages, mast cells, and neutrophils all exhibit considerable plasticity in response to microenvironmental stimuli [41]. The term "trained immunity" has been used to describe the immunologic memory of human innate immunity [44]. BCG vaccination of healthy human volunteers strongly enhanced subsequent in vitro production of monocyte-derived cytokines such as tumor necrosis factor α and interleukin 1β in response to unrelated bacterial and fungal pathogens [47]. BCG-induced immune enhancing effects persisted for at least 3 months after vaccination, and were accompanied by increased expression of monocyte CD11b and TLR4. These innate training effects were induced through NOD2 and mediated by increased histone methylation. Further support for innate training effects come from an animal model: *SCID* mice, which have no T and B lymphocytes, were protected against disseminated candidiasis by BCG vaccination [47]. Epigenetic reprogramming by vaccines, leading to alteration of gene methylation, acetylation, and chromatin structure that modulate gene expression, may thus be a key candidate mechanism underlying vaccine heterologous effects, including long-lasting effects on disease susceptibility. Gene reprogramming can alter immune development including Th1/Th2 balance [48] and Th17 and regulatory T-cell profiles [49, 50]. Early age-related changes in DNA methylation profiles of mononuclear cells from healthy infants support the effect of environment on epigenetic programming in early life [51]. This emerging area warrants investigation in the context of infant vaccination.

SEX DIFFERENCES IN IMMUNOLOGIC RESPONSES TO VACCINES

There are clear sex differences in responses to childhood and adult viral vaccines. In general, females have stronger humoral responses and higher rates of adverse reactions [52], consistent with greater activation of innate and adaptive immune responses in females following exposure to pathogens, allergens, immunogens, and toxins. Unfortunately, the underlying mechanisms have barely been studied but, given the overall proinflammatory effects of low-dose estradiol, and the anti-inflammatory effects of testosterone and progesterone, sex hormone–mediated effects may well play a role [53]. Indeed, there are hormone response elements present on antiviral genes, and sex hormone receptors are expressed on many immune cells [54]. Furthermore, many immune-regulated genes are expressed on the X chromosome [55], which may escape X inactivation, allowing greater gene expression among females. Similarly, X-linked microRNAs may have immunoregulatory functions that can be modulated by sex hormones. Gambian infant studies showed changes in reactivity to TLR2, 4, 5, and 7/8 four weeks after vaccination with MV or DTwP, with males consistently having higher reactivity than females, suggesting that MV and DTwP can alter the setpoint and polarization of TLR-mediated cytokine production in a sex-differential manner. In vitro reactivity to recall antigens was similarly higher in males than females 4 weeks after MV or DTwP vaccination, and whole-genome transcriptional profiles were highly dependent on sex, with females differentially expressing many more genes than males. Many of these effects were lost when MV and DTwP were coadministered (F. Noho-Konteh, U. J. Adetifa, T. Forster, M. Cox, D. Jeffries, M. T. Le, F. Barker, A. Drammeh, J. Njie-Jobe, H. C. Whittle, S. Rowland-Jones, P. Dickinson, P. Ghazal, and K. L. Flanagan, unpublished data). A sex-differential vaccine response was also noted for yellow fever vaccination, again with females upregulating many more genes [52]. Overall, these data highlight the value of analyzing outcomes of vaccine trials by sex and of understanding the effects of coadministering vaccines and other health interventions, as often occurs for logistic reasons.

HETEROLOGOUS ADAPTIVE IMMUNITY AND VACCINE HETEROLOGOUS EFFECTS

Heterologous T-cell immunity could provide a further mechanism whereby vaccines protect against infections that are not targeted by the vaccines [56]. Virus-specific T-cell responses can be robust in neonatal mice, even in the presence of circulating maternal antibodies, but may have a narrower T-cell repertoire with a distinct immunodominance hierarchy compared to adult mice [57]. T-cell–based heterologous immunity is well described in mice; for instance, infection with lymphocytic choriomeningitis virus primes cross-reactive immunity against Pichinde virus and vaccinia virus [58]. These heterologous effects can lead to beneficial protective cross-reactive responses or cause harmful immunopathology, and are distinct in males and females. Heterologous immunity is also observed in humans where reactivation of influenza A–specific memory CD8 T cells can be either detrimental or beneficial upon Epstein-Barr virus infection, dependent on the private specificity of the cross-reactive influenza A–specific memory population in each individual [59]. It will be important to characterize human T-cell epitopes for human vaccine antigens and common childhood pathogens to enhance the study of heterologous T-cell immunity in children.

EFFECT OF GEOGRAPHIC LOCATION ON VACCINE RESPONSES

Vaccine responses can vary depending on whether children live in urban vs rural areas [60]; high- vs low-socioeconomic urban areas [61]; or the developing (Malawi) vs developed (United Kingdom) world [62–64]. Potential causes for population differences include genetic variability and environmental factors, including intercurrent infections such as helminths [64], nutrition, and microbiome. Rural populations had greater messenger RNA expression of IL-10, programmed cell death protein 1, and immunoglobulin E than urban populations; and rural children had a higher ratio of lipopolysaccharide-induced phosphorylation of monocyte-derived extracellular signal-regulated kinases to p38 mitogen activated protein kinase than urban children, suggesting a Th2 skew (M. Yazdanbakhsh, A. Amoah, and S. de Jong, unpublished data). Therefore, vaccine studies should consider the potential confounding factors of geographical location and local environmental differences when studying human populations.

CONCLUSIONS

A growing body of evidence from diverse fields of immunology, developmental ontogeny, vaccinology, and epidemiology suggest that vaccines have substantial heterologous effects on the development of the immune system, and susceptibility to a range of unrelated pathogens. These heterologous effects vary by age, sex, and environment and may be amplified by micronutrient supplements. These observations have major implications for basic and translational vaccine research and may also eventually guide vaccine policy. The Optimmunize group concluded that basic, translational, and clinical studies of vaccine heterologous effects and their sex-differential nature are major research priorities. State-of-the-art tools to investigate the immune system, including transcriptomics, metabolomics, tissue engineering, microbiomics, epigenomics, multiparameter flow cytometry, and phosphosignaling should be used to study the heterologous effects of vaccines to elucidate underlying biologic mechanisms. Independent replication of the randomized epidemiologic trials from West Africa is a high priority, and researchers should take every opportunity to characterize immunologic mechanisms.

It is hoped that characterizing the mechanisms underlying vaccine heterologous effects will not only enable reduction of any potentially untoward vaccine heterologous effects, but also allow beneficial heterologous effects to be harnessed through deliberate, timed, and targeted immune modulation. This novel approach affords an exciting opportunity to leverage newly discovered heterologous effects to optimize vaccine schedules and thereby substantially enhance the benefits provided by immunization programs, potentially resulting in further dramatic reductions in child mortality.

Notes

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