



Conference report

Sex differences in the vaccine-specific and non-targeted effects of vaccines

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ABSTRACT

Vaccines have non-specific effects (NSE) on subsequent morbidity and mortality from non-vaccine related infectious diseases. Thus NSE refers to any effect that cannot be accounted for by the induction of immunity against the vaccine-targeted disease. These effects are sex-differential, generally being more pronounced in females than males. Furthermore, the NSE are substantial causing greater than fifty percent changes in all cause mortality in certain settings, yet have never been systematically tested despite the fact that millions of children receive vaccines each year. As we strive to eliminate infectious diseases through vaccination programmes, the relative impact of NSE of vaccines on mortality is likely to increase, raising important questions regarding the future of certain vaccine schedules. A diverse group of scientists met in Copenhagen to discuss non-specific and sex-differential effects of vaccination, and explore plausible biological explanations. Herein we describe the contents of the meeting and the establishment of the 'Optimmunize' network aimed at raising awareness of this important issue among the wider scientific community.

1. Introduction

There are accumulating data illustrating that males and females may respond differently to vaccination, both in terms of the quality and quantity of the immune response. If true, then we must consider whether vaccination schedules should differ for males and females, or as has been suggested “should we treat the sexes differently in order to treat them equally?” This would require a major paradigm shift, and the scientific community would need to be convinced before this could happen. A workshop was held in Copenhagen in January 2010 to discuss the current evidence, speculate on the underlying biological mechanisms, and drive forward a research agenda to investigate this important issue.

2. Setting the scene

The driving force behind the workshop, and two major proponents of the hypothesis that males and females differ in their responses to vaccines, are Prof. Peter Aaby and Dr. Christine Benn, based at the Statens Serum Institut, Copenhagen and Bandim Health Project, Guinea-Bissau. Their extensive vaccine studies in Guinea-Bissau and Senegal in West Africa have consistently found sex-differential effects on the outcome of childhood vaccination [1–9]. Interrogation of datasets from a number of vaccine studies throughout the world further confirm differences between males and females in response to vaccination [10–14].

The background originates from studies that show that vaccines have non-targeted non-specific effects (NSE) on morbidity and all-cause mortality from subsequent infectious diseases. By NSE, we are referring to any effect that cannot be accounted for by the induction of immunity against the vaccine-targeted disease. Overall, live vaccines such as Bacillus Calmette-Guérin (BCG) and measles vac-

cine (MV) provide beneficial effects, whereas inactivated vaccines such as the diphtheria, tetanus, pertussis (DTP) combined vaccine can have deleterious effects on survival in high mortality settings such as Guinea-Bissau. The NSE, which differ according to sex, can be substantial with changes in all-cause mortality of greater than fifty percent. For example, in randomised trials in West Africa, high-titre measles vaccine (HTMV) increased female mortality two-fold between 4 months and 5 years of age, with no corresponding deleterious effects in boys [15]. The introduction of DTP in rural areas of Guinea-Bissau increased mortality of girls two-and-half-fold in the subsequent 6 months [16]. Girls randomised to vitamin A supplementation in Ghana had two-and-half fold higher mortality if they received DTP during follow-up whereas boys receiving vitamin A had lower mortality than placebo recipient [13].

The order in which vaccines are received is critical, as data reveal that the last vaccine given appears to determine the NSE outcome. For example, the increased female mortality after HTMV could be explained by the subsequent administration of DTWP [15], and DTWP given either with or after measles vaccination cancels the beneficial effects of the measles vaccine [12]. These described effects are of enormous public health importance, yet have never been systematically tested despite the fact that millions of children receive vaccines each year according to recommended Expanded Programme on Immunization (EPI) schedules.

The findings were originally met with considerable skepticism, with some regarding Aaby and Benn as modern day heretics. However, analysis of data from many observational studies and a few relevant trials from countries including Gambia [12], Senegal [11], Ghana [13], Benin [17], Sudan [10], Malawi [14], Congo [10], Haiti [18] and Bangladesh [19] have all supported NSE on all-cause mortality. The hypothesis that vaccines have NSE is now becoming more widely accepted, and, indeed, only non-targeted effects can explain

the fact that BCG is used as a treatment for bladder cancer [20]. A meeting of experts convened at the London School of Hygiene and Tropical Medicine (LSHTM) in 2008 to discuss vaccine NSE concluded that there is sufficient scientific evidence that they exist, with three publications emerged from the meeting [21–23].

The sex-differential nature of these NSE further fuels the controversy, and adds another layer of complexity to this intriguing issue. A diverse group of scientists with expertise in epidemiology, endocrinology, evolutionary biology, basic and applied immunology, molecular biology, nutrition and reproductive biology met to generate interdisciplinary cross-talk, discuss the scientific evidence and consider the way forward. The primary aim of the meeting was to consider the mechanisms mediating sex differences in the NSE of vaccines. The following report details the content and outcome of the meeting.

3. Epidemiological evidence

Peter Aaby outlined the epidemiological evidence for NSE and sex differential effects of vaccines. Christine Benn went on to present compelling evidence that vitamin A supplementation can amplify these NSE in a sex differential manner [3,9,13], suggesting that the World Health Organization (WHO) policy of providing vitamin A supplementation at the time of childhood vaccination might need to be revisited. The data suggest that females derive little benefit from vitamin A supplementation when administered at the same time as the DTP vaccine. Aaby and Benn provided a list of >100 peer reviewed scientific studies (available on request) dating back almost 30 years, many published in general medical journals, including *The Lancet* and *The British Medical Journal*, all supporting sex-differential and NSE of vaccines and vitamin A supplementation. They challenged the workshop participants to identify plausible biological mechanisms for their controversial findings.

4. Sex differences in disease susceptibility and vaccine responses

Sabra Klein, from The Johns Hopkins Bloomberg School of Public Health, USA presented data demonstrating that the intensity and prevalence of infection with viruses, bacteria, and parasites are higher in male humans and mice than their female counterparts, which likely involves physiological as well as behavioural differences between the sexes [24,25]. In particular, females mount higher innate and adaptive immune responses to pathogen challenge than do males [26,27]. Although heightened immunity in females can be beneficial for reducing viral load and clearing viruses, these immune responses can be detrimental if they become too robust or remain elevated for too long, leading to the development of 'immunopathology'. Data from HIV and influenza patients were presented to support this paradox (i.e. lower viral loads, yet more severe disease in females compared with males) [28,29]. Indeed, Klein et al. have recently published a paper describing more robust innate responses in females to the yellow fever vaccine as well as higher antibody responses in females to the influenza vaccine, combined measles, mumps, rubella (MMR) vaccine, and hepatitis A and B vaccines [30]. In addition to having higher immune responses, females also experience more adverse events following vaccination. These data illustrate that sex differences in response to vaccination are evident in both adults and children.

5. Innate immunity

The innate immune response refers to non-specific defense mechanisms that occur rapidly (within minutes or hours) follow-

ing encounter with antigen. This should not be confused with NSE of vaccines, which may be caused by a variety of mechanisms including innate immune cell activity. The innate immune response is only recently being investigated as a key component of the immune response to vaccination, yet many vaccine components or co-administered adjuvants elicit inflammatory innate responses. These occur via activation and binding to pattern recognition receptors (PRRs), such as Toll-like receptors (TLRs) and NOD receptors, and prime the antigen presenting functions of innate immune cells, including dendritic cells and macrophages. For example, polio, measles, yellow fever and BCG vaccines all contain intrinsic TLR agonists [31], and there is presently considerable interest in developing TLR agonists as vaccine adjuvants [32].

Infants are born with minimal adaptive memory and rely on their innate response in early life [33]. However, the newborn has poor reactivity to innate immune stimuli, such as TLR ligands [34], and a defect in the MyD88 signaling pathway has been described at birth [35]. Ian Marriott, from the University of North Carolina at Charlotte, USA presented data suggesting sex differences in innate immune responses. An overactive innate immune response is largely responsible for the poor outcome in bacterial septic shock. Components of bacteria activate PRRs, and this activation results in more severe disease in both human and rodent males compared to females. For example, in response to LPS, male mice generally produce more pro-inflammatory cytokines, such as IL-6 and TNF- α , whereas macrophages from females produce more of the anti-inflammatory cytokines (reviewed in [36]). Furthermore, estrogens increase and testosterone decreases the expression of the LPS receptor TLR4 on immune cells [37,38]. Thus, the effects of reproductive hormones on innate immunity may contribute to the early life sex-differential NSE of vaccines.

6. Adaptive immunity

Despite the fact that many infants receive in excess of 20 vaccines in the first 9 months of life, relatively little is understood about the development of immunity in this age group. Adaptive immunity describes the generation of memory B and T cells that are specific for a particular antigen and can be recalled upon re-exposure to that antigen. The adaptive cellular immune response to vaccination remains poorly characterized by sex in humans in general, and infants in particular, and analyses for NSE of vaccines on immune reactivity are absent. T cell memory responses are primed and boosted by vaccination, with live vaccines generally eliciting helper T cell type 1 (Th1) responses, and killed vaccines with aluminium adjuvants generally induce helper T cell type 2 (Th2) reactivity. Infant dendritic cells have defective IL-12 production, which decreases Th1 priming [39], and may contribute to Th2-biased immunity in early life [40,41]. However, BCG vaccination at birth can stimulate an adult-like Th1 response [42] alongside a Th2 response, although the Th1 response may wane by 9 months while the Th2 response is sustained [43]. Similarly measles vaccination primes a predominant Th1 response in infants and children [44].

Katie Flanagan, from the MRC Laboratories, The Gambia, presented data on the immunological effects of DTP and MV on cytokine profiles to a range of vaccine-specific and non-specific antigens in Gambian infants, which illustrate that reactivity to live and killed vaccines is more complex than a simple Th1/Th2 dichotomy. Furthermore, immune reactivity in her studies seems to be quite different in males and females, with 10 month old males producing higher levels of a wide range of cytokines and chemokines to vaccine and non-vaccine antigens compared to age-matched females, and males having higher frequencies of circulating memory T cells following vaccination than

females (Flanagan et al., unpublished data). Further detailed studies of the contribution of the adaptive immune response to the NSE of vaccination in early life need to be performed, including use of state-of-the-art technologies such as genomics and proteomics. The signature gene and protein expression profiles within the adaptive immune response pathways might identify potential factors contributing to NSE.

7. Hormonal differences in infancy

Niels Skakkebaek, from the University Department of Growth and Reproduction, Rigshospitalet, outlined the differences in the hypothalamic–pituitary–gonadal axis in boys and girls in early life. He pointed out that infant males have an early gonadotropin and testosterone surge during the first 6 months which peak at 2–3 months. In females the pattern of gonadotropins is rather heterogeneous, although infant females generally have higher oestrogen levels than males in this age group [45,46].

Many immune cells, including T cells, B cells, macrophages, neutrophils, dendritic cells, and natural killer cells, have sex hormone receptors. Sex hormones can affect the differentiation and activation of these immune cells via signaling through their receptors, and may contribute to the sex-differential NSE of vaccination. Mogens Claesson, from the Department of International Health, University of Copenhagen, further emphasized that the sex hormone milieu in early life might affect responses to vaccines by altering the balance between Th1, Th2 and regulatory T cell responses. Thus, differences in sex hormone levels could account for sex differences in immunological responses to vaccines in early life, which should be systematically explored.

8. X-linked immune response genes

Eleanor Fish, from the University Health Network and the University of Toronto, pointed out that many immune response genes are X-linked and thus subject to X-inactivation in females, while only one copy is universally expressed in males. This immediately provides one plausible explanation for sex differences in immune responses to vaccines. The X-linked genes include immune response related proteins, cytokine receptors, TLRs and transcriptional and translational effectors (reviewed in [47]). The relative contribution of X-linked immune response genes to NSE of vaccines is an unexplored area. Moreover, Prof. Fish commented that constitutively higher levels of circulating T cells in females compared with males, also likely contribute to sex-differential NSE of vaccines.

9. Vitamin A

Andrew Prentice, from The London School of Hygiene and Tropical Medicine, UK, discussed the evidence for the immunological effects of vitamin A supplementation. Macrophages convert vitamin A into retinoic acid (RA) which promotes Th2 immunity, inhibits Th1 and Th17 differentiation, induces FOXP3⁺ regulatory T cells, and regulates B cell proliferation and differentiation (reviewed in [48]). The Bill and Melinda Gates Foundation and WHO have jointly commissioned three neonatal vitamin A epidemiological trials in India, Ghana, and Tanzania with mortality as an endpoint, and three additional mechanistic studies, including human studies in The Gambia and Bangladesh that will investigate how co-administration of high dose vitamin A at birth influences innate and adaptive immune responses to vaccine and non-vaccine antigens. The immunomodulatory effects of vitamin A may affect the immune system following vaccination in a number of ways, and these studies may shed light on the mechanisms whereby vita-

min A amplifies the sex differential NSE of vaccines. **Main points**• Vaccines have non-specific effects (NSE) on morbidity and mortality from non-vaccine related infectious diseases• The NSE are substantial with >50% changes in subsequent all-cause mortality• The order in which vaccines are given is critical in relation to NSE• NSE are sex-specific with females generally being affected more than males **Suggested areas of further research**• Large randomised controlled trials of sex-specific morbidity / mortality following vaccination• Systematic characterization of innate and adaptive immune responses to vaccination by sex• The role of sex hormones in the immune response to vaccination• The role of X-linked immune response genes as mediators of sex differences in response to vaccines• The mechanisms whereby vitamin A amplifies the NSE of vaccines• Standardization of methods to measure vaccine reactogenicity• The priming of heterologous immune responses by specific vaccines

10. Vaccine inflammation and reactogenicity

Arnaud Marchant, from the Université Libre de Bruxelles, Belgium, pointed out that vaccines are well known to induce local and systemic inflammatory reactions aside from stimulating the classic innate signaling pathways. Indeed, by their very nature vaccines are designed to elicit an inflammatory response. Inflammation is a complex process involving a variety of mediators including cytokines, chemokines, lipid mediators, complement activation, proteolytic enzymes and vasoactive amines and peptides. Alum is the most widely used human vaccine adjuvant, and may activate the inflammasome, although this remains controversial [49,50]. The adjuvant MF59 (a squalene emulsion) has specifically been shown to induce cytokine and chemokine transcription in mouse muscle. This response could involve a direct interaction between the adjuvant and non-immune tissue cells [51]. Clinical trials in adults reveal that women respond more strongly and experience more adverse reactions to vaccine adjuvants than men [52]. Genetic and proteomic studies suggest that adverse reactions to smallpox vaccination are associated with prolonged stimulation of inflammatory pathways and an imbalance of normal tissue damage repair pathways [53]. A number of serum cytokine mRNAs and proteins, including ICAM-1, G-CSF, IL4 and IL-10, were identified as correlates of systemic reactogenicity. Standardization of methods to measure reactogenicity will help in exploring the mechanisms involved and the role of specific inflammatory mediators. Marchant suggested that inflammation and reactogenicity should be a target for studies of the adverse effects of vaccines, and may contribute to both non-specific and sex-specific vaccine effects.

11. Heterologous immunity

Heterologous immunity is the process whereby exposure to one pathogen or antigen alters the immune response/susceptibility to a different pathogen or antigen. An important distinction is that heterologous immunity describes a distinct mechanism involving the generation of cross-reactive immune memory whereas NSE of vaccines may be caused by heterologous immunity or other mechanisms. Liisa Selin, from the University of Massachusetts, discussed the way in which heterologous boosting might explain some of the NSE of vaccines. Her data illustrate that in mice, primary infection with influenza A virus, followed by vaccinia virus (VV) challenge decreases pathology when compared to mice that received VV alone. In contrast, infection with influenza virus followed by LCMV results in increased pathology when compared to mice that received LCMV alone [54,55]. The induction of cross-reactive T cells by the primary infection results in differential protection

against subsequent challenge. T cell cross-reactivity is common between unrelated pathogens and alters T cell immunodominance in sequential or simultaneous infections. T cell cross-reactivity can alter the efficiency of the effector response and thus influence protective immunity and immunopathology; the size of the T cell cross-reactive response correlates with severity of immune pathology. It can also lead to narrowing of the T cell repertoire, giving rise to viral escape mutants, and non-cross-reactive T cells can be deleted during the course of an infection resulting in a loss of memory.

The priming of cross-reactive immune responses could explain both beneficial and harmful vaccine effects. Mogens Claesson, from the Department of International Health, University of Copenhagen, proposed a model whereby live vaccines prime antigen presenting cells (APCs) leading to beneficial innate responses to unrelated pathogens [56]. The cytokines and chemokines released by the activated APCs would determine the nature of the immune response to the unrelated pathogen. He further suggested that DTP vaccination might induce myeloid-derived suppressor cells leading to generalized immunosuppression and increased susceptibility to certain pathogens [57]. An inactivated vaccine might also induce cross-reactive responses that cause increased immunopathology or deviate the immune system in other harmful ways. Liisa Selin's more recent studies show male–female differences in heterologous immunity in visceral fat tissue of mice (*unpublished data*). The mechanisms whereby heterologous immune priming by vaccination leads to altered susceptibility to non-vaccine related diseases, and whether this is sex-differential, needs to be explored in the context of the described NSE of vaccines. This would be a fascinating area of research particularly because heterologous immunity, being epitope specific, could feasibly be manipulated in vaccine design.

12. Evolutionary perspective

Jacobus Boomsma, Head of the Centre for Social Evolution, University of Copenhagen, provided an evolutionary perspective on the issue of sex-differential effects. He pointed out that adaptive immunity has evolved as a compromise to maximize survival and reproduction, and that adaptation is phenotypically plastic rather than 'hard-wired'. He suggested that human populations may be adapted to low birth weight and nutritional deficiency in resource poor settings, and that developmental trade-offs *in utero* may leave males and females differently vulnerable. He speculated that the mortality issues may not arise in a high-income setting in the absence of the poor hygiene and nutrition. However, the first studies of NSE in high-income countries have found beneficial effects of BCG and smallpox vaccination in both males and females [58,59].

13. The research priorities: a call to action

Sex differences in the NSE of vaccines is an area of major public health importance that remain largely unexplored. Indeed, several articles in *Nature* recently highlighted the fact that females are generally under-represented in biomedical studies, and analysis by sex is rarely performed on data from clinical trials [60,61]. There are a number of plausible biological mechanisms by which non-specific and sex-specific effects might occur, but the relative contribution of each requires investigation. It is possible that particular vaccines exert NSE through distinct mechanisms, and given the differences between infant and adult immunity it is likely that mechanisms of NSE in infants may not apply to the adult immune system, although additional studies are required. Given the preliminary data that the chronology of vaccine administration affects morbidity, and that these effects segregate according to sex and are amplified by vitamin A, the working hypotheses are (1) that the chronology of

antigen (vaccine/pathogen/vitamin A) exposure durably influences immune programming and the subsequent immune response to unrelated pathogens, and (2) that sex-based differences *a priori* affect immune responses.

14. Detailed immunological studies

Detailed immunological studies of the vaccine-specific and NSE of vaccines on the innate and adaptive arms of the immune system need to be carried out on datasets that are large enough and sufficiently powered to be analysed by sex. State-of-the-art technologies including genomics, proteomics and metabolomics might also be applied to answer the question of how vaccines mediate NSE differentially in males and females.

15. Large randomised trials

There is an urgent need to confirm in randomised controlled trials that vaccines have significant NSE on all-cause mortality which may vary by sex [23]. It is also important to confirm the observed interactions between vaccines and vitamin A. Much of the data have arisen from studies in Guinea-Bissau and the results need to be reproduced in different settings.

16. Analyses of existing datasets by sex

It is probable that numerous scientists already have datasets that could be re-analysed by sex. We appeal to anyone reading this paper to consider whether they have such data, particularly from vaccine studies, that might be re-analysed in this way. This would then highlight the issue of sex-differential effects in immune responses to vaccines, and add to the paucity of publications that specifically describe sex-differential immunological responses. An example of such a reanalysis of existing data was presented by Wafei Fawzi's group [62]; maternal multivitamin supplementation had a sex-differential effect on subsequent child mortality, being beneficial for girls but not for boys.

17. 'Optimmunize': a research network to examine sex differential effects of vaccines

The working group has established an international consortium called 'Optimmunize' to investigate the NSE and the sex-differential effects of vaccines. Attempts will be made to raise international scientific awareness, and drive forward the much needed studies required for the biological mechanisms to be understood and exploited in vaccine implementation programmes. If vaccines can be shown conclusively to have NSE on morbidity and mortality, and the sex differences and their mechanisms are confirmed, this will have major public health implications and raise the imperative issue of whether males and females require different vaccine schedules.

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