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Sex difference in immune response to vaccination: A participant-level meta-analysis of randomized trials of IMVAMUNE® smallpox vaccine

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ABSTRACT

Introduction: Previous research shows immune response to vaccination differs by sex but this has not been explored for IMVAMUNE®, a replication-deficient smallpox vaccine developed in response to the potential for bioterrorism using smallpox.
Methods: We conducted a participant-level meta-analysis ($N = 275$, 136 men, 139 women) of 3 randomized trials of IMVAMUNE conducted at 13 centers in the US through a federally-funded extramural research program. Studies were eligible for inclusion if they tested the standard dose (1×10^8 $TCID_{50}$/mL on Days 0 and 28) of liquid formulation IMVAMUNE, were completed at the time of our search, and enrolled healthy *vaccinia*-naïve participants. Models of the peak log_2 ELISA and PRNT titers post-second vaccination were constructed for each study with sex as a covariate. Results from these models were combined into random effects meta-analyses of the sex difference in response to IMVAMUNE. We then compared this approach with fixed effects models using the combined participant level data.
Results: In each study the mean peak log_2 ELISA titer was higher in men than women but no single study demonstrated a statistically significant difference. Combination of the adjusted study-specific estimates into the random effects model showed a higher mean peak log_2-titer in men compared with women (absolute difference [men–women]: 0.32, 95% CI: 0.02–0.60). Fixed effects models controlling for study showed a similar result (log_2 ELISA titer, men–women: 0.34, 95% CI: 0.04–0.63). This equates to a geometric mean peak titer that is approximately 27% higher in men than women (95% CI: 3–55%). Peak log_2 PRNT titers were also higher (although not significantly) in men (men–women: 0.14, 95% CI: −0.30 to 0.58).
Conclusion: Our results show statistically significant differences in response to IMVAMUNE comparing healthy, vaccinia-naïve men with women and suggest that sex should be considered in further development and deployment of IMVAMUNE and other MVA-based vaccines.

1. Introduction

The potential to prevent infectious diseases (ID) through vaccination was recognized in the late 18th century, although it wasn't until the 19th and 20th centuries that vaccination significantly impacted public health [1–3]. Advances in knowledge of pathogens and Omics, studies of pathogen–host interactions, and mechanisms of immunity have improved the efficiency and success of vaccine development [1,4,5]. However, the fundamental assumption underlying vaccine deployment has remained largely unchanged, i.e., that a single vaccine for a given pathogen can be used in a large population [6]. This assumption is incongruous with contemporary recognition that the immune response is heterogeneous and that a single vaccine may have varying utility in population subgroups [7].

Heterogeneous post-vaccination immune responses in men and women have been reported across a range of vaccines and in populations with different characteristics [8]. The effect of sex on immune response to vaccination may depend on several factors, including the vaccine antigen itself, with men responding better to some antigens than women and vice versa [8]. Recent studies of military personnel and civilian healthcare workers vaccinated against smallpox using Dryvax vaccine showed that females

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maintain stronger long-term humoral immunity than males [9], but that sex differences in cellular immune response are less consistent, with the female (or male) dominance depending on individual cytokines [10]. The possibility that population subgroups respond differently to smallpox vaccination is of concern given the development of novel smallpox vaccines intended for emergency use against a bioterrorist attack with weaponized smallpox [11].

Vaccines against smallpox are based on the poxvirus *vaccinia*, which induces immunity against *variola* virus, the causative agent of smallpox [12]. Unfortunately, live-virus vaccines such as Dryvax, which were successfully used to eradicate smallpox, are associated with rare but potentially fatal adverse events, e.g., disseminated vaccinia and myopericarditis [13], and the newly developed vaccinia-virus smallpox vaccine ACAM2000®, which is now licensed in the US for limited use in people at risk, is associated with similar safety concerns [14]. IMVAMUNE® is a highly attenuated smallpox vaccine developed as a safer alternative to existing live virus vaccines. IMVAMUNE is based on the Modified Vaccinia Ankara (MVA) virus, which is a replication-deficient *vaccinia* virus, first experimented with in the 1970s as a priming agent intended to reduce adverse reactions of subsequent vaccination with live *vaccinia* virus vaccines [15]. The possibility of bioterrorist attack using smallpox generated renewed interested in MVA as a smallpox vaccine and this led to development of IMVAMUNE, which is now licensed by the European Medicines Agency and Health Canada for prevention of smallpox and continues to be tested in clinical trials in the United States [15–17].

Sex differences in response to IMVAMUNE have not been explored, and although men and women have been included in randomized trials of IMVAMUNE, individual trials were not powered to detect differences in immune response between sexes. Therefore, we conducted a participant-level meta-analysis of completed randomized trials of IMVAMUNE to evaluate sex differences in humoral immune response to this novel smallpox vaccine. Our objective was to inform the design of future studies of IMVAMUNE and other MVA-based vaccines, and to explore the importance of sex in human immunity in general.

2. Methods

Our approach to conducting and reporting this analysis followed established standards for meta-analysis of clinical trials [18,19].

2.1. Identification of studies

Since 2002, the Division of Microbiology and Infectious Diseases (DMID) at the National Institute of Allergy and Infectious Diseases (NIAID) has sponsored clinical trials of IMVAMUNE through its extramural research program. During February of 2014, in collaboration with DMID/NIAID staff, we identified all DMID-sponsored clinical trials of IMVAMUNE for which participant-level data were available at the DMID/NIAD data coordinating center (The EMMES Corporation, Rockville, MD). We then selected studies for our meta-analysis from this portfolio.

2.2. Eligibility criteria

Studies eligible for inclusion in our meta-analysis of sex differences in humoral immune response to IMVAMUNE were: randomized clinical trials (these studies offer high-quality evidence that IMVAMUNE elicits a humoral immune response), completed at the time of our search (required for extraction of results), included healthy participants only (to exclude effects of established pathological processes on immune function), enrolled participants who were naïve to smallpox vaccine (to exclude the effect of immunological experience on immune response to IMVAMUNE), and tested the liquid formulation of IMVAMUNE in the standard dose, 1×10^8 $TCID_{50}$/ml via subcutaneous needle injection on Days 0 and 28 (chosen because this formulation, dose, and administration timing elicits the strongest humoral immune response) [20].

2.3. Data extraction

Participant-level data were obtained for each of the included studies. We extracted data for all participants from each study who received two doses (on Days 0 and 28) of liquid formulation IMVAMUNE at 1×10^8 $TCID_{50}$/mL. Data were not extracted for participants receiving placebo or other IMVAMUNE regimens. Included studies measured humoral immune response at various time points after each vaccination. We focused our analysis on measurements taken after the second vaccination as this is the time period when IMVAMUNE is shown to elicit the strongest humoral immune response [20]. Antibody titers, measured by enzyme linked immunosorbent assay (ELISA) and plaque reduction neutralizing titer (PRNT), were extracted from each included study for several time points post-second vaccination (Table 1). The primary endpoint for our meta-analysis was the highest $\log_2$-transformed titer achieved for each individual, which we interpreted as an estimate of the peak titer. We chose the mean difference in the $\log_2$-transformed titer, comparing men to women, as the summary measure for our meta-analysis as this allowed us to assess both the presence and magnitude of the sex difference in response to IMVAMUNE. This method also allowed us to interpret the anti-logarithm of the sex difference as a relative measure of geometric mean titer in men vs. women.

2.4. Statistical analysis

We began by plotting the mean of the $\log_2$-titer over time by sex, separately for each study. This allowed us to qualitatively evaluate the presence of sex differences in response to IMVAMUNE prior to conducting any formal analyses. Our meta-analysis of the peak $\log_2$-titer followed a two-stage approach, in which we estimated the sex difference in response to IMVAMUNE in each study separately (the first stage) and then combined the study-specific results into a random effects model (the second stage) using the method described by Der Simonian and Laird [21]. For each study, we applied the generalized linear modeling framework, with identity link and normal errors, to estimate the absolute difference in peak $\log_2$-titer between men and women. We selected four models for each study: an unadjusted and adjusted model of the peak $\log_2$-titer as measured by ELISA and PRNT. Selection of adjusted models was performed by exploring the importance of the following factors, one at a time, in a model containing sex: age (continuous and categorical), study center, $\log_2$-titer immediately prior to second vaccination, and race (White vs. non-White). Any factors that were significant (alpha = 0.10) after controlling for sex, or that substantially improved model fit over a model containing only sex (as indicated by the Akaike or the Bayseian information criteria) were entered simultaneously into a model containing sex. Then, backward elimination was applied until all predictors in the model (other than sex) were significant at alpha = 0.05. All models used females as the referent group. Statistical significance was evaluated using the likelihood ratio chi-square test, and model assumptions were verified graphically. The coefficient and standard error for sex were extracted from each of the final models for incorporation into random effects models. Finally, we evaluated the impact of inter-study heterogeneity on the summary estimate of the sex difference in $\log_2$-titer by fitting joint fixed effects models (i.e., treating all of the participant-level data as if it were collected as part of a single study) and informally comparing results with the random effects models. Joint fixed effects models were

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