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Preliminary evidence that morning vaccination is associated with an enhanced antibody response in men.

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Running Head: Timing of vaccination and antibody response.

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Abstract

Variation in response to vaccination, particularly in vulnerable groups, provides a strong rationale for developing vaccine adjuvants. If there were consistent diurnal variation in immune response this could inform a simple intervention for enhancing vaccine efficacy. Data from two studies are presented examining morning versus afternoon vaccine administration; in the first, hepatitis A vaccine was administered to young adults, and in the second, influenza vaccine to older community-based adults. Men, but not women, vaccinated in the morning mounted a better peak antibody response to both hepatitis A and the A/Panama influenza strain. These results indicate that it would be worthwhile testing this effect in a large randomised control trial with vaccination during time periods representing the extremes of hormonal and cytokine diurnal rhythms.

Descriptors**:** antibody response; diurnal; vaccination;

Vaccination is a valuable strategy to reduce morbidity and mortality from a variety of diseases. Most modern non-replicating purified subunit or synthetic viral vaccines have limited immunogenicity on their own (Edelman, 1980), resulting in inadequate antibody production and disease protection, particularly among vulnerable groups such as older adults (Hodes, 1997). Consequently, substantial resource has gone into researching vaccine adjuvants; usually aluminium compounds which can give rise to adverse side effects (Gupta & Siber, 1995). Even with adjuvant help, there is considerable variation in vaccination response (Jefferson, 2006). This could arise from a variety of vaccine-related factors, such as dose (Lynn, et al., 2004) and delivery needle size (Diggle, Deeks, & Pollard, 2006). However, less attention has been paid to the circumstances surrounding vaccine administration, such as time of day. In an early study, individuals given an attenuated Venezualan equine encephalomyelitis vaccine at 8am exhibited peak antibody titres four days earlier than those vaccinated at 8pm (Feigin, Jaeger, McKinney, & Alevizatos, 1967), whereas administration of a hepatitis B vaccine in the afternoon between 1 and 3pm induced higher antibody levels than morning injections between 7.30 and 9 am (Pollman & Pollman, 1988). Finally, a study of influenza vaccination in this context reported that the peak response to the A/Philippines strain occurred when the vaccine was administered between 11.00am and 1.00pm (Langlois, Smolensky, Glezen, & Keitel, 1995). Taken together, these preliminary results provide little guidance as to the best time to vaccinate. Achieving a better immune response to vaccination is clinically important, particularly for disease susceptibility and mortality in older adults (Crofts, et al., 2003). Accordingly, if consistent diurnal variations in antibody response to vaccination were to emerge from further study, this could inform a simple and realisable means of improving disease protection (Petrovsky, McNair, & Harrison, 1998). Consequently, we present analyses of the impact of time of day of vaccination on antibody response from two separate studies. The first study assessed the response to a hepatitis A vaccine in young healthy adults, and the second examined responses to the annual influenza vaccination in older community-based adults.

Method

Participants

In Study 1, participants were 75 (34 men) University of Birmingham students with a mean age of 22.9 (*SD* = 3.89) years. In terms of ethnicity, 89% described themselves as "white," 2.7% as "Asian," 1.3% as "black," and 7% as "other". None of the participants were

suffering from a current acute infection, chronic medical condition, or immune disorder. Ninety-five percent reported being non-smokers. For Study 2, data were available for 89 older adults (38 men) recruited from five medical practices in Birmingham, UK for a study on the associations between psychosocial factors and the antibody response to influenza vaccination, reported elsewhere (Phillips, et al., 2006). All participants were aged 65 years or older and had no acute infection or known current immune disorder. Participants' mean age at entry to the study was 73.1 (*SD* = 5.49) years. All but one described themselves as "white": one described themself as "black", and one as "other". Ninety-one per cent were non-smokers.

Procedures

At an initial session, participants provided a venous blood sample before vaccination to determine baseline antibody status. In study 1, participants were randomly allocated to either a morning (10am to 12pm; n = 39) or early evening (4pm to 6pm; n = 36) vaccination session; although given academic timetabling practicalities, about 30% of participants could only attend a specific session. They were vaccinated with the hepatitis A vaccine (HAVRIX™; Glaxo SmithKline). In study 2, the baseline session was when older adults were attending their medical practice for the annual influenza vaccination. Time of day of vaccination was therefore an opportunistic variable. These data were collated following completion of the study using appointment records and the researcher's own files. A binary am/pm variable was created; to provide a clear temporal division yet retain sufficient power to detect effects, participants vaccinated between 11am and 1pm were excluded. Fifty-nine participants vaccinated in the morning between 8am and 11am, and 30 in the afternoon between 1pm and 4pm. The 2003-04 influenza vaccine, administered by a nurse, contained three viral strains: A/New Caledonia/20/99; A/Panama/2007/99; and B/Shangdong/7/97. In both studies the follow-up blood sampling took place approximately one month later (younger adults mean = 31.3; SD = 3.10 days; older adults mean = 28.6; SD = 2.91 days).

Sample preparation and immunological assays

Serum was assessed for hepatitis A antibody titres using a commercial quantitative assay, Enzygnost® Anti-HAV (Dade Behring, Germany). Anti-influenza antibody titers were measured by the serology laboratory of Glaxo Smith Kline Beecham at Dresden, Germany,

using a haemagglutination inhibition test as described in the World Health Organisation Manual on animal influenza diagnosis and surveillance.

Data analysis

Antibody titres were $\log_{10}$ transformed due to their marked variability. To examine time of day effects on antibody response, ANCOVA was applied using the log antibody titer at one-month follow-up to each antigen as the dependent variable. Time of day of vaccination was entered as a fixed factor. Given that two previous studies by our group have observed sex differences in the response to vaccination (see Edwards, et al., 2006; Phillips, Burns, Carroll, Ring, & Drayson, 2005), sex was also analysed as a fixed factor. Baseline titre was entered as a covariate. Effect sizes are reported as η^2. High negative life events exposure in the hepatitis A study was associated with a poorer vaccination response (Gallagher, Carroll, & Phillips, 2007), so the total number of life events was added as a covariate in subsequent analyses of the younger sample. Since older age and having experienced bereavement in the past year had a negative effect on antibody response in the older sample (Phillips, et al., 2006), age and bereavement served as further covariates in subsequent analyses. Finally, whether or not participants mounted a two-fold increase in antibody titre from baseline was calculated to provide an estimate of the clinical implications of any diurnal variation in vaccine response.

Results

Vaccination response

Participants responded with a significant increase in antibody titre for all four antigens from baseline to one month (see Table 1).

Associations between time of day of vaccination and antibody response

For hepatitis A, there was no time of day or sex main effect on antibody response. There was, however, a significant sex x time of day interaction, $F(1,70) = 6.74$, $p = .01$, $\eta^2 = .088$: men exhibited a better antibody response to a morning than an afternoon vaccination, whereas women tended, if anything, to mount a better antibody response to the afternoon vaccination. In the older adults, our analyses again failed to detect significant time of day or sex main

effects on the response to any of the three viral strains. However, for A/Panama, there was a sex x time of vaccination interaction effect, $F(1,84) = 5.93$, $p = .02$, $\eta^2 = .066$: as before, men, but not women, mounted a better antibody response to morning vaccination.

Associations between time of day of vaccination and antibody response adjusting for covariates

The above analysis for hepatitis A was repeated adjusting for total life events exposure. Again, there was a significant sex x time of day interaction, $F(1,69) = 6.56$, $p = .01$, $\eta^2 = .087$. In the older adults, repeat analysis included age and whether or not the participant had experienced bereavement as covariates. The sex x time of vaccination interaction for A/Panama remained significant, $F(1,82) = 7.12$, $p = .009$, $\eta^2 = .080$. These effects are illustrated in Figure 1 using estimated marginal means.

Sex differences in time of day and vaccination response associations

In order to examine the sex x time of day interaction effects further, separate ANCOVAs were conducted for men and women. For the response to hepatitis A, the difference in antibody response with time of day of vaccination was statistically significant only in the men, $F(1,31) = 5.86$, $p = .02$, $\eta^2 = .159$. For the influenza A/Panama strain in the older adults, men again responded better in the morning, $F(1,35) = 5.45$, $p = .03$, $\eta^2 = .135$. These correspond to medium to large effects and remained significant following adjustment for the relevant covariates. Although women appeared to show better antibody responses following afternoon vaccination, the difference between morning and afternoon in women was not statistically significant, for hepatitis A, $F(1,38) = 1.81$, $p = .19$, $\eta^2 = .045$, and A/Panama, $F(1,48) = 0.84$, $p = .36$, $\eta^2 = .017$. For hepatitis A, 87% of men vaccinated in the morning showed a two-fold response, in comparison to 47% of men vaccinated in the afternoon. For influenza, 41% of men showed a two-fold response to the A/Panama strain when vaccinated in the morning versus 24% of men vaccinated in the afternoon.

Discussion

Previous research on the effects of time of day of vaccine administration on antibody response is limited and inconsistent (Feigin, Jaeger, McKinney, & Alevizatos, 1967; Langlois, Smolensky, Glezen, & Keitel, 1995; Pollman & Pollman, 1988). Data from the current

studies consistently demonstrated an interaction between sex and time of day of vaccination; men vaccinated in the morning exhibited a stronger antibody response to hepatitis A and the A/Panama influenza strain than men vaccinated in the afternoon. Almost twice as many men showed a two-fold increase in antibody titre when vaccinated in the morning as opposed to the afternoon. Of the three strains that make up the influenza vaccine, A/Panama appears to be particularly susceptible to exogenous influence (see Edwards, et al., 2006; Phillips, Burns, Carroll, Ring, & Drayson, 2005; Phillips, et al., 2006). The mechanism underlying this effect remains unclear. However, cytokines and cortisol exhibit diurnal rhythmicity (Petrovsky, McNair, & Harrison, 1998), and both are potent regulators of immune function (Cooper, Duckett, Petts, & Penny, 1979; Dhabhar, 2002). Although there is little evidence of sex differences in such diurnal rhythms (Edwards, Evans, Hucklebridge, & Clow, 2001; Hansen, Garde, Christensen, Eller, & Netterstrom, 2001), it is possible that cytokines and cortisol variations have different consequences for immunity in men and women.

The present study has several limitations. First, participants were not fully randomised to morning or afternoon vaccination sessions, making it difficult to discount completely alternative explanations for the effects found. For example, it is possible that men attending the earlier vaccination sessions were less distressed or, in the influenza study, were also younger. However, it is difficult to see why bias of this sort would be specific to men. In addition, adjustment for measures of stress in both samples and age in the older sample did not change the outcomes. Further, for the majority of participants in the older sample, time of appointment was not optional but was allocated by their medical practice, and in the younger sample allocation was as random as possible within academic timetable constraints. Nevertheless, residual confounding by either unmeasured factors or variables measured with error must remain a possibility (Christenfeld, Sloan, Carroll, & Greenland, 2004). Second, as the older sample analysed here were part of a larger study examining the effects of psychosocial factors on vaccination, precise data on time of vaccine administration were not collected for all participants, meaning that only a dichotomous am/pm variable could be sensibly derived. This variable is clearly a crude reflection of specific timing of vaccination. Nevertheless, the introduction of time-specific vaccination as an intervention would need to be compatible with the organisation of health services. In this context the contrast of a morning-based with an afternoon-based service has some ecological validity. Analyses still yielded significant temporal effects on antibody response for men when the younger sample were grouped by hour of vaccination and when the group vaccinated between 11am and 1pm in the older sample was included. However, the sample sizes in these finer grain analyses

were such (N = 7 in some cases) that we are reluctant to draw inferences beyond our conclusion that it would appear to be better for men to be vaccinated earlier rather than later. Third, although we have posited that a differential impact of cortisol or cytokine variations on men and women's immunity may underlie the present findings, in the absence of data such explanations are necessarily speculative.

Our findings should be regarded as tentative. Nevertheless, the effect sizes were medium to large in statistical terms. At a population level, titre variations of this magnitude could have clinical significance. At the very least, our results suggest that a large scale properly randomised control trial is worth pursuing. If these effects were replicated, they would have major implications for the temporal scheduling of vaccination programmes and could provide a very cost effective vaccine adjuvant.

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Table 1. *Geometric Mean (95% Confidence Intervals) Antibody Titers at Initial Baseline and at the One Month Follow-up.*

Viral Strain	Pre-vaccination	One-month Follow-up	ANOVA (p<.001)
Hepatitis A	37.15 *(2 – 575)*	199.53 *(23 – 1698)*	$F(1,74) = 166.66$ $p < .001$, $\eta^2 = .693$
A/New Caledonia/20/99	38.02 *(4-407)*	64.57 *(8-537)*	$F(1,88) = 24.96$ $p < .001$, $\eta^2 = .221$
A/Panama/2007/99	56.23 *(4-724)*	95.50 *(8-1122)*	$F(1,88) = 36.39$ $p < .001$, $\eta^2 = .293$
B/Shangdong/7/97	57.54 *(5-631)*	104.71 *(11-955)*	$F(1,88) = 40.76$ $p < .001$, $\eta^2 = .317$

Figure 1: Associations between time of day of vaccination and antibody response.