The time course of the inflammatory response to the *Salmonella typhi* vaccination

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ABSTRACT

The *Salmonella typhi* vaccination induces transient increases in inflammatory-responsive cytokines and molecules. For instance, it causes small, mild increases in interleukin-6 (IL-6) within a few hours and C-reactive protein (CRP) within 24 h. No study has charted either the time course of the inflammatory response to this vaccine or any associated changes in mood, physical symptoms, and cardiac function. In a blinded crossover experimental design, eight participants received the *S. typhi* vaccine (vaccination condition) and a saline (control condition) injection on two separate days, at least one week apart. Blood samples and mood ratings were collected at 0, 4, 5, 6, 7, 8 and 24 h post-injection, physical symptoms and pain were assessed at 4–8 and 24 h post-injection, and cardiovascular function was recorded until 8 h post-injection. Repeated measures analyses of variance and polynomial trend analyses compared the timecourse of the response patterns between the two conditions. Whereas there were no temporal changes in the control condition, the vaccination increased granulocytes, IL-6, TNF-α, and CRP (all p's < .05). Specifically, the granulocytes, IL-6 and TNF-α peaked after 6–8 h while CRP peaked after 24 h. This vaccine-induced mild inflammatory response was not accompanied by any changes in mood or cardiovascular activity. We also found that participants tended to report more pain in the injected limb in the vaccination condition (p < .07). In sum, our study charted the timecourse of key inflammatory-responsive markers following *S. typhi* vaccination and identified the timing of their modest peaks. It is worth noting that changes in these markers were not accompanied by any notable changes in mood or cardiovascular activity, and thus the *S. typhi* vaccination is a suitable method to induce increases in inflammatory-responsive markers, without altering mood or cardiovascular parameters.

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1. Introduction

Inflammation is an adaptive response of the immune system to infection and tissue damage (Medzhitov, 2008). Two characteristic features of this response are rapid tissue infiltration by leukocytes and the release of inflammatory-responsive cytokines (Medzhitov, 2008). Acute inflammation is deemed functional and protective whereas a chronic inflammatory response is generally considered dysregulated and harmful (Medzhitov, 2008; Stevens et al., 2005). Indeed, chronic inflammation has been implicated in the aetiology of a range of diseases, such as cardiovascular disease and its complications (Hansson, 2005; Levy et al., 2008; Libby, 2006; Ridker et al., 2000). In addition, inflammation is also suspected to play an aetiological role in cognitive dysfunction and psychopathology. For example, patients exhibiting elevated inflammatory activity, such as acute coronary syndromes (Lesperance et al., 2004) and auto-immune diseases like rheumatoid

arthritis (Zautra et al., 2004), show increases in dysphoric mood and posses a substantially elevated risk for the development of clinical depression and anxiety. While this is often used to suggest a role of inflammation in mood disorders, interpretation of such patient data is confounded by other biological (e.g., medications) and psychological risk factors (Cleland et al., 2000; Vita et al., 2004). Therefore, experimental approaches have been explored whereby the effects of inflammatory activity can be studied in healthy participants.

Two experimental manipulations frequently used to induce transient systemic inflammation involve the administration of low dose *Escherichia coli* endotoxin and vaccination against *Salmonella typhi*. The latter pathogen is the cause of typhoid fever and one of the main sources of food poisoning. The benefits of using the *S. typhi* vaccination is that it is approved by regulatory bodies (e.g., US Food and Drug Agency) and does not typically induce fever or feelings of malaise (Hingorani et al., 2000; Strike et al., 2004; Wright et al., 2005), which is in contrast to endotoxin (Reichenberg, 2001). However, mild unpleasant side effects, like aching joints, headache and nausea, to typhoid vaccination have been

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noted in some (Wright et al., 2005) but not other (Brydon et al., 2009) studies.

Typhoid vaccination reliably induces a mild systemic inflammatory response: increases in IL-6 have been documented within 2 h (Brydon et al., 2009) and are sustained up to 12 h (Antoniades et al., 2011) post vaccination. There is less consistent evidence for TNF-α, with some studies reporting increases at 4 h post vaccination (Kharbanda et al., 2002), and others not finding any changes (Hingorani et al., 2000; Wright et al., 2005). Increases in the acute phase protein CRP are consistently seen at 24 h post vaccination (Antoniades et al., 2011; Padfield et al., 2010). However, no prior study has charted the time course of these responses, and it is therefore unknown when the peak responses are reached and how long they remain elevated. Finally, physical symptoms accompanying vaccine-induced inflammation have typically been assessed only at one or two time points, and, therefore, the time course of physical symptomatology is also unknown.

A similar story can be told about the effects of typhoid vaccination on mood, which has yet to be fully characterized. Studies that administered endotoxin found transient increases in negative mood states, such as anxiety and depression (Eisenberger et al., 2009, 2010; Reichenberg, 2001), whereas studies that injected S. typhi vaccination reported more equivocal effects. One study reported an increase in negative mood following vaccination, but this effect only became apparent after the ratings were contrasted with a placebo control condition that was associated with an elevated positive mood; hence, these data indicated the absence of positive mood induction rather than the presence of negative mood (Strike et al., 2004). Another study observed positive correlations between changes in IL-6 and negative mood at 3 h post-vaccination, such that greater IL-6 responses were associated with more negatively-valenced feelings (Wright et al., 2005). In contrast, there was no increase in negative mood within 2 h after vaccination (Brydon et al., 2009). Therefore, it is likely that changes in mood occur only when the inflammatory response has had more than a couple of hours to develop. A time course study is required to resolve this issue. Although one study has examined the time course of mood in response to vaccination, it did not provide data on the inflammatory response and its links with mood and physical symptoms (Strike et al., 2004).

Based on the putative role of inflammation in cardiovascular pathology, typhoid vaccination has been utilized in studies of the inflammation–cardiovascular function relationship. Studies have yet to demonstrate any effects of vaccination on blood pressure, heart rate, cardiac output, and total peripheral resistance (Chia et al., 2003; Padfield et al., 2010; Strike et al., 2004; Vlachopoulos et al., 2005). However, using lipopolysaccharide as a stimulus, one study observed reductions in heart rate variability (Kox et al., 2011), which is consistent with the literature on the bi-directional regulation between the inflammatory system and the vagal nerve (Andersson and Tracey, 2012). It is therefore surprising that no studies have examined the influence of vaccine-induced inflammation on cardiovascular indices of vagal, or for that matter sympathetic drive, particularly given preliminary evidence that decreased heart rate variability may be a potential mechanism linking depression with elevated cardiac mortality in patients who have suffered a myocardial infarction (Carney and Freedland, 2009; Taylor, 2010).

Grounded on the literature reviewed above, the present study was designed to characterize the inflammatory response to S. typhi vaccination over a 24 h period. No study to date has reported the time course of inflammatory, psychological (mood, symptoms) and physiological (cardiovascular) responses to vaccination, and, therefore, our findings should improve our understanding of the effects of vaccination in humans. It was anticipated that the vaccine would induce systemic inflammation (as reflected by increases in

circulating leukocytes, TNF-α, IL-6, and CRP), increases in negative mood, and reduced heart rate variability. The effects on self reported physical symptoms were also explored, however, due to lack of prior evidence, no specific predictions were made.

2. Methods

2.1. Participants

Eight ($N = 8$) male participants (mean age ± SD = 26.63 ± 8.07 years, mean body mass index ± SD = 23.21 ± 1.80 kg/m^2) were recruited. At the time of testing, none were suffering from an acute illness or infection, reported a history of inflammatory, cardiovascular or auto-immune disorders, or had taken any medication in the last 4 weeks. None had vaccine-related allergies or had received a typhoid vaccination in the last 12 months. Participants reported to the laboratory having refrained from vigorous exercise for at least 24 h, from alcohol for at least 12 h and food or caffeine in the 2 h prior to the start of testing. The study was approved by the local research ethics committee, and all participants gave written informed consent.

2.2. Procedure

Participants completed an inflammatory condition (vaccination) and a control condition (saline) on two separate days, at least 7 days apart, in a counter-balanced blinded design. All participants reported to the laboratory between 8:30 and 9:00 am, and after written consent was obtained, questionnaires were completed and instrumentation for cardiovascular assessment undertaken. Blood pressure was recorded before the first blood sample was taken (0 h). Next, the injection was administered and participants waited 20 min in the presence of a registered nurse to check for any adverse events. A blood sample, questionnaires, and cardiovascular measurements were taken at regular time points (i.e., 4 h, 5 h, 6 h, 7 h and 8 h post-injection). A final measurement was taken the following day, 24 h after injection, when participants returned to the laboratory. The protocol's timing and measurements were identical in both conditions. Participants were asked to replicate their diet on the subsequent testing day in an attempt to control for the effects of diet upon the responses to vaccination. There was at least a 45 min period between a meal and a blood sample.

2.3. Vaccination

On each day, participants received one of two injections: 0.5 ml S. typhi capsular polysaccharide vaccine (0.025 mg in 0.5 ml, Typhim Vi, Sanofi Pasteur, UK) or a saline placebo (0.5 ml) via intra-muscular injection into the deltoid muscle of the non-dominant arm, in a counterbalanced, blinded crossover design.

2.4. Questionnaires

Subjective ratings of physical responses to vaccination and mood were assessed by questionnaire at 4 h, 5 h, 6 h, 7 h, 8 h and 24 h post-injection. Participants were asked to rate the presence and severity of physical symptoms on a checklist, using a 5-point Likert scale, with anchors of 0 (not at all) and 5 (severe). These symptoms included malaise, sore throat, watery eyes or eye strain, upset stomach, sneezing, congested nose, persistent cough, headaches, neck ache, shortness of breath, feverishness, chills, nasal discharge, and ringing in the ears. Participants were also asked to rate pain in their joints and pain at the site of the injection using a 10-point scale, with anchors of 0 (not at all) and 10 (very severe). The ratings for the general symptoms and local symptoms were

aggregated separately to give an overall score for each class of symptoms at each time point. The Cronbach alpha coefficients for the physical symptoms and pain ratings at the site of injection ranged from .79 to .96, indicating good internal consistency of the measures.

Mood was assessed with a short form of the Profile of Mood States (POMS) questionnaire (McNair et al., 1981), from which the following 6 constructs were calculated; tension-anxiety, anger-hostility, vigor-activity, fatigue-inertia, confusion-bewilderment, and depression-dejection. Participants were asked to rate on a 5-point scale (0 = not at all, 4 = extremely), how they felt at that precise moment. The six POMs constructs were averaged at each time point. Total negative mood was calculated by combining all negative constructs together as described elsewhere (Wright et al., 2005). The Cronbach alphas for each subscale ranged from .43 to .98 in the saline condition and from .68 to .96 in the vaccination condition. There was one low reliability score in the saline condition, which was due to the absence of feelings of anger; the remaining coefficients were above .65.

2.5. Physiological measurements

2.5.1. Cardiovascular measures
Resting blood pressure was assessed at each blood draw using an Omron 705CP (HEM-705CP-E) blood pressure monitor. Systolic blood pressure (SBP) and diastolic blood pressure (DBP) were used to estimate mean arterial pressure (MAP). Indices of cardio-dynamic activity were recorded continuously using the Vrije Universiteit Ambulatory Monitoring System (VU-AMS, Amsterdam, The Netherlands) (de Geus et al., 1995; Willemsen et al., 1996). This system used six Ag/AgCl spot electrodes (Invisatrace, ConMed Corporation) to record electrocardiography (ECG) and impedance cardiography (ICG), in line with published guidelines (Sherwood et al., 1990). Sixty-second ensemble averages were calculated and used to determine Heart Rate (HR, bpm), Pre-Ejection Period (PEP; ms) and Root Mean Square of Successive Differences (rMSSD, ms) as a measure of Heart Rate Variability (HRV).

2.5.2. Blood sampling
The blood draws at baseline (0 h) and 24 h were taken using a 21 gauge butterfly needle (Becton Dickinson, UK). The remaining draws (4–8 h) were taken using a cannula (18 gauge, Insyte, Becton Dickinson), which was inserted into an antecubital vein in the participant's dominant arm. The timing of cannula insertion remained consistent across both conditions. On each draw, the first 3 ml of blood was collected in a syringe and discarded. Blood was collected into two 4 ml and one 2 ml vacutainers containing potassium ethylene diaminetetraacetic acid (K3EDTA) (Becton Dickinson, UK). The 4 ml samples were stored on ice until centrifugation (1500×g for 10 min at 4 °C) and plasma was stored at −80 °C for later assessment of IL-6, TNF-α, and CRP. The 2 ml samples were placed on a roller until analyzed for full blood cell count (total lymphocyte, monocyte and granulocyte count) using a Coulter Analyzer (Beckman Coulter, Inc.).

2.5.3. Assays
Plasma IL-6 and TNF-α were measured in duplicate using high-sensitivity ELISAs (Quantikine HS Human IL-6 ELISA and Quantikine HS Human TNF-α ELISA, both R&D Systems, UK) in accordance with the manufacturer's instructions. The reported sensitivity of the assays was 0.039 pg/ml and 0.106 pg/ml for IL-6 and TNF-α respectively, with recorded intra-assay and inter-assay variations both <10%. Analysis of high sensitivity C-reactive protein was undertaken at a commercial laboratory (Synlab, Leinfelden, Germany) by immunonephelometry using a Behring Nephelometer II. The detection limit for CRP was 0.015 mg/l (High Sensitivity CRP,

Dade Behring), with all samples assayed in the same run, yielding a within-assay CV% of <4.5%.

2.5.4. Data reduction and analysis
A series of 2 Condition (Control, Vaccination) by 7 Time (0, 4, 5, 6, 7, 8 and 24 h post-injection) within-subjects repeated measures analyses of variance (ANOVAs) were conducted on the inflammatory and mood measures. A series of 2 Condition (Control, Inflammation) by 6 Time (0, 4, 5, 6, 7, and 8 h post-injection) repeated measures ANOVAs were performed on the physiological responses (HR, PEP, rMSSD, and MAP). For all ANOVAs, the Greenhouse-Geisser epsilon (ε) correction was applied where appropriate (Vasey and Thayer, 1987). Given that our study aimed to chart the temporal patterning of our measures, we also conducted polynomial trend analyses to compare the time course of the response to *S. typhi* vaccination and saline injection for each inflammatory, physiological and psychological measure. Eta squared (η^2) was used as a measure of effect size. Occasional missing data are reflected in the reported degrees of freedom.

3. Results

3.1. Inflammatory response to vaccination
Fig. 1 illustrates the inflammatory response to vaccination in terms of changes in the numbers of lymphocytes, monocytes and granulocytes as well as the concentrations of IL-6, TNF-α and CRP. The 2 Condition × 7 Time ANOVAs yielded overall condition by time interaction effects for granulocytes ($F(1, 6) = 12.07$, $p = .002$, $\varepsilon = .50$, $\eta^2 = .50$) and IL-6 ($F(1, 7) = 11.24$, $p = .042$, $\varepsilon = .51$, $\eta^2 = .32$), with a trend for monocytes ($F(1, 6) = 1.09$, $p = .098$, $\varepsilon = .42$, $\eta^2 = .30$). To characterize the time course of the inflammatory response in the vaccination and placebo conditions, we computed polynomial trend analyses on the immune variables. These analyses revealed condition by time quadratic effects for monocytes ($F(1, 6) = 13.06$, $p = .011$, $\eta^2 = .69$), granulocytes ($F(1, 7) = 14.73$, $p = .006$, $\eta^2 = .68$) and IL-6 ($F(1, 7) = 6.00$, $p = .044$, $\eta^2 = .46$). In addition to the condition by time interaction effects, the expected time effects for granulocytes, lymphocytes and IL-6 (p's < .05), as well as condition effects for granulocytes and IL-6 (p's < .05) were also observed. As can be seen in Fig. 1, monocytes, granulocytes, and IL-6 only increased following vaccination, peaking around 6–7 h post-inoculation, before returning to baseline levels after 24 h.

While the initial analyses yielded no overall condition, time, or condition by time effects (p's > .10), the polynomial trend analyses revealed significant condition by time interaction effects for both hsCRP and TNF-α (see Fig. 1). The CRP responses were characterized by a significant condition by time linear effect ($F(1, 7) = 8.48$, $p = .027$, $\eta^2 = .59$), due to a gradual increase in CRP in the vaccination condition, peaking after 24 h post vaccination, and a gradual decrease in CRP in the control condition. The TNF-α responses were characterized by a significant condition by time cubic effect ($F(1, 7) = 6.92$, $p = .034$, $\eta^2 = .50$), due to increases in TNF-α in the vaccination condition, plateauing 6–8 h post vaccination, accompanied by decreases in TNF-α in the placebo condition.

3.2. Physical symptoms in response to vaccination
Analysis of the physical symptom and pain reports (see Table 1) revealed no condition by time effects and no time effects (p's > .10). The condition main effect was not significant for physical symptoms whereas pain at the site of injection tended to be elevated in the vaccination condition compared to the control condition ($F(1, 7) = 4.77$, $p = .065$, $\varepsilon = 1.00$, $\eta^2 = .41$).
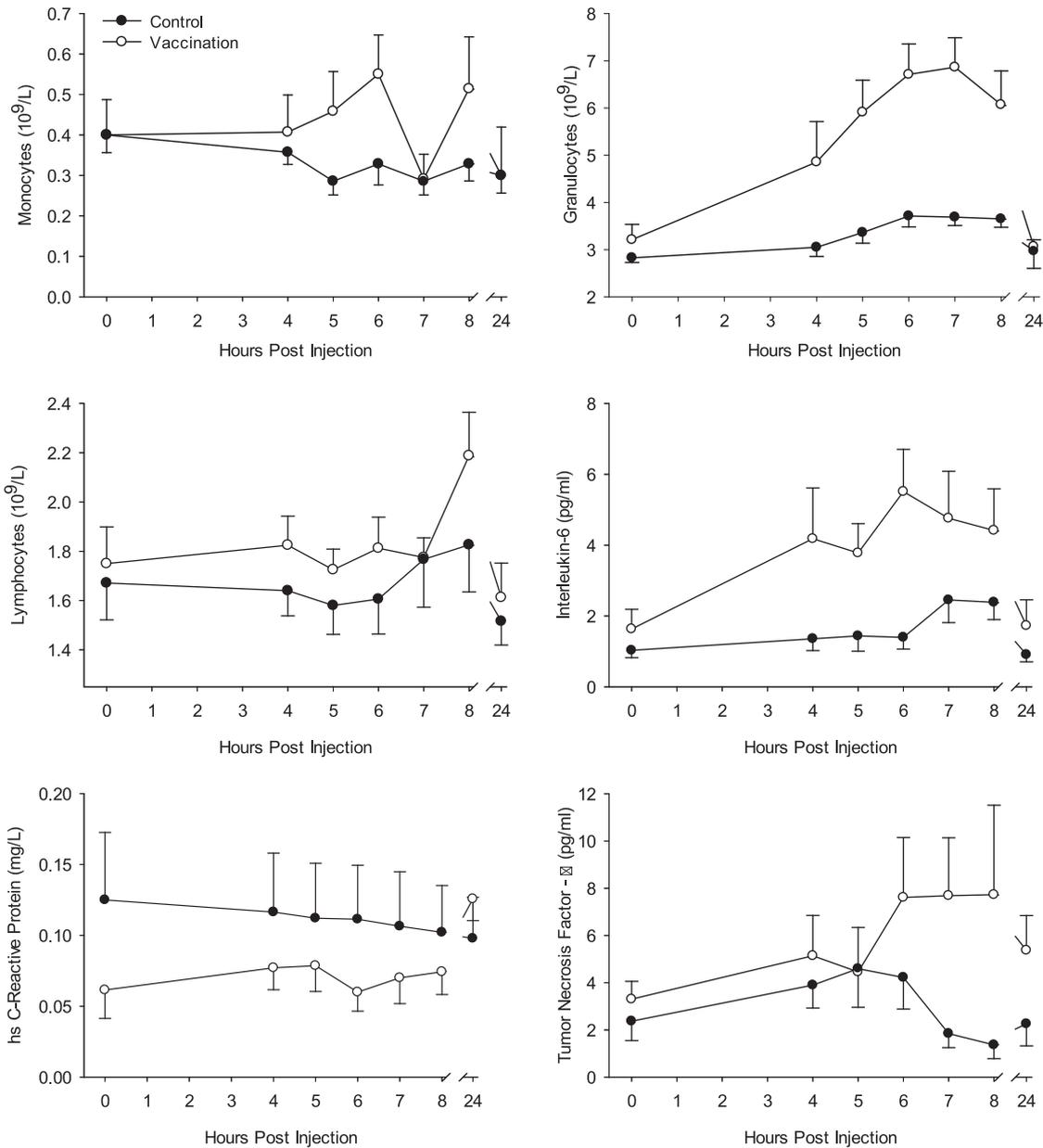
Fig. 1. Mean ± SE monocyte, lymphocyte, hsCRP, granulocyte, IL-6, and TNF-α responses to vaccination. Filled circles represent the control condition, whereas clear circles represent the vaccination condition.

Table 1
Mean (SD) physical symptoms in response to vaccination on a scale of 0–10 for physical symptoms, and 0–5 for pain.

Time post-injection (hours)	Physical symptoms (0–10)		Pain (0–5)	
	Control condition	Vaccination condition	Control condition	Vaccination condition
4	0.13 (0.35)	0.75 (1.49)	0 (0)	1.06 (0.82)
5	0 (0)	0.50 (1.41)	0 (0)	1.56 (1.97)
6	0.25 (0.71)	0 (0)	0 (0)	1.14 (1.89)
7	0.13 (0.35)	0 (0)	0 (0)	0.88 (0.92)
8	0 (0)	0 (0)	0 (0)	1.00 (1.71)
24	0.25 (0.71)	0.13 (0.35)	0 (0)	1.25 (2.02)

Note: For the physical symptom reports, a score of 0 indicates 'Not at all', with 10 indicating 'Severe'. For the pain responses, a score of 0 indicates 'Not at all', with 5 indicating 'Severe'. Aggregated physical symptoms refer to the aggregate of all physical symptoms, with pain referring to localized pain at the site of vaccination.

3.3. Physiological responses to vaccination

Fig. 2 captures the time course of HR, PEP, rMSSD, and MAP in the hours following injection. It is notable that there were no significant condition by time or condition effects (all p's > .10).

3.4. Psychological responses to vaccination

The levels of negatively-valenced moods experienced by participants in the two conditions are depicted in Fig. 3. A series of 2 condition × 7 time ANOVAs was used to examine each individual
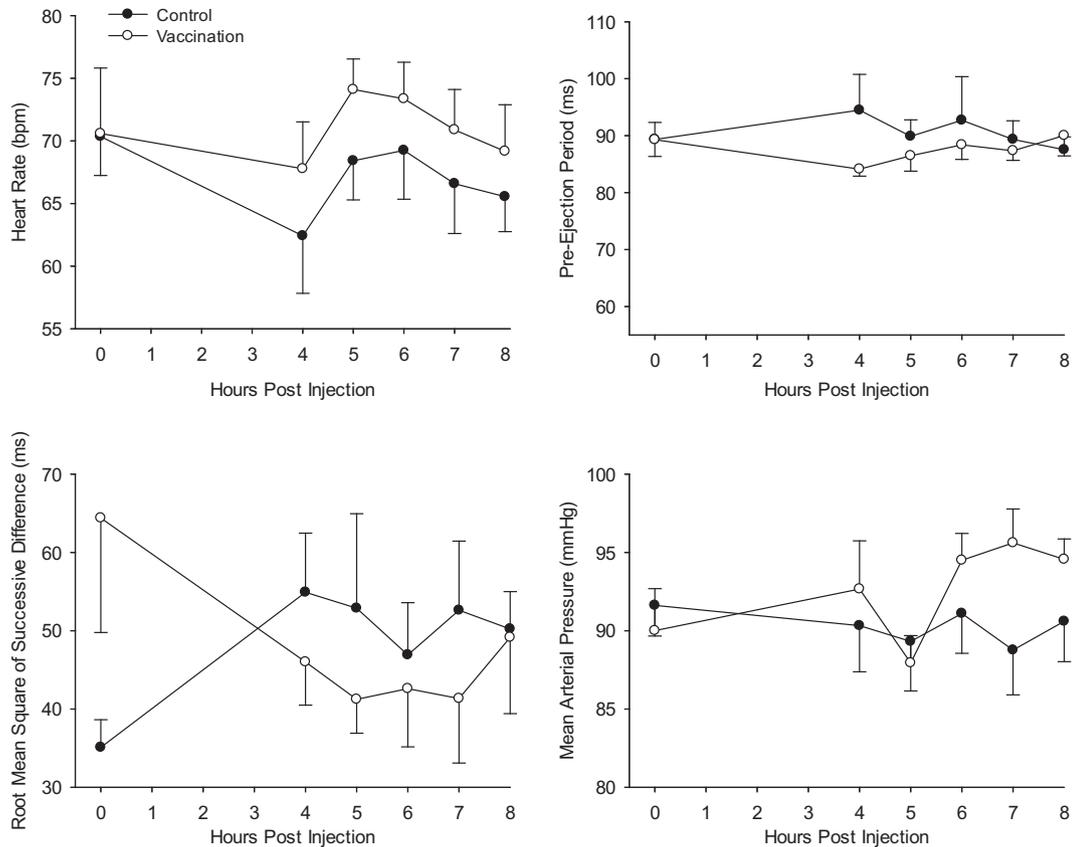
Fig. 2. Mean ± SE cardiovascular responses to vaccination for HR, rMSSD, PEP and MAP. Filled circles represent the control condition, whereas clear circles represent the vaccination condition.

negative mood construct (tension, anger, fatigue, confusion, and depression) and the total negative mood score (which was computed by averaging the scores of the five individual negative mood constructs). These analyses yielded no condition, time, or condition by time interaction effects (p's > .10). Thus, there were no differences in mood between conditions and no changes in mood over time in either condition.

4. Discussion

The present study aimed to characterize the time course of the inflammatory, physical, mood and cardiovascular responses to a

Fig. 3. Mean ± SE total negative mood ratings over the 24 h period post-injection, in both the control (filled circles) and vaccination (open circles) conditions.

typhoid vaccination. This study was the first, to our knowledge, to compare the time course of the inflammatory response to a vaccination and saline injection over a 24 h period. In support of our predictions, the administration of a 0.5 ml dose of the S. typhi vaccine increased the concentrations of two cytokines, IL-6 and TNF-α, as well as the numbers of other immune cells in the blood, with responses peaking (at more than double the initial values) approximately 6–7 h post vaccination and returning back to basal levels at 24 h post vaccination. CRP levels showed only a small rise in the first 8 h, but increased to more than double the basal levels after 24 h post vaccination. In addition, participants reported slightly more pain at the site of the injection following vaccination. However, no effects of vaccination on mood and cardiovascular activity were observed.
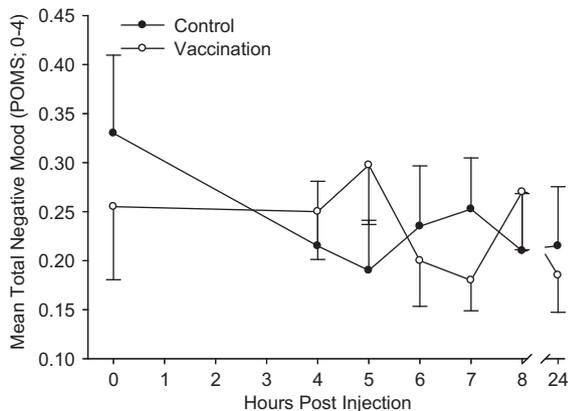
4.1. Effects of vaccination on inflammatory markers

This study provides new information about the time course of the inflammatory response to a 0.5 ml dose of the S. typhi vaccination in comparison to a saline injection over a 24 h period. The magnitude and the timing of the increases in inflammatory markers observed in our study are consistent with other studies that have used a typhoid vaccine. Increases in IL-6 following vaccination have been demonstrated at 2 h (Brydon et al., 2009), 3 h (Wright et al., 2005), 4 h (Kharbanda et al., 2002), 6 h (Chia et al., 2003; Padfield et al., 2010), 8 h (Antoniades et al., 2011; Clapp et al., 2004; Ekstrom et al., 2008; Vlachopoulos et al., 2007) and 12 h (Antoniades et al., 2011) post vaccination. One study examined the response to a double dose (i.e., 1 ml) of the vaccination used in the current and most other studies, revealing the peak IL-6 response to be at 10 h post-vaccination (Bennermo et al.,

2004). The larger quantity of vaccine might be one reason for their peak IL-6 response being delayed, and given that more antigen would have been within the vaccine, a more substantial and prolonged inflammatory response is likely to have occurred.

Levels of TNF-α changed following vaccination in a similar pattern to IL-6 in this study. The observed increases in TNF-α might have been a consequence of the macrophage response to vaccination, which tends to precede the release of IL-6 (Möller and Villiger, 2006). There appears to be great variability in the TNF-α response to *S. typhi* vaccination, with studies reporting increases (Kharbanda et al., 2002) and no changes (Harrison et al., 2009; Hingorani et al., 2000; Wright et al., 2005). Possible reasons for these discrepancies include differences in the timing of the blood draws and the amount of vaccine administered (e.g., 1 ml versus 0.5 ml) (Bennermo et al., 2004; Hingorani et al., 2000; Kharbanda et al., 2002; Wright et al., 2005). Trend analyses showed that CRP increased selectively at 24 h in the vaccine condition, which is in line with other studies (Antoniades et al., 2011; Padfield et al., 2010) and is considered part of the normal time course of this acute phase protein, which typically shows the strongest rise 12–24 h after induction of inflammation. Given the relationship between IL-6 and CRP (Ekstrom et al., 2008; Möller and Villiger, 2006), it likely that the increases in CRP observed in the vaccination condition are the result of increases in other pro-inflammatory cytokines, such as TNF-α and IL-6, in response to vaccination. Consequently, it is not unreasonable to suggest that the typhoid vaccination may act as a useful model to study some aspects of elevated inflammatory activity, but, admittedly, not others (e.g., consequences of immune perturbations seen during more severe inflammatory diseases).

The current study found no significant condition differences in reported physical symptoms. This null finding is in line with previous research demonstrating that *S. typhi* vaccination does not induce feelings of malaise or fever (Hingorani et al., 2000; Strike et al., 2004; Wright et al., 2005), or aching in the limb in which the vaccination was administered (Brydon et al., 2009). However, we found that participants in the vaccination condition tended to report more localized pain in the limb that was injected compared to the saline condition. This tendency may be due to the relatively small changes in physical symptoms which have also been reported elsewhere (Wright et al., 2005). Alternatively, given the large effect size ($\eta^2 = .41$), the small sample size (and low statistical power) in the current study may also have contributed to the condition effect for reported pain being only marginally significant (Cohen, 1992). Taken together, our results suggest that, in addition to the systemic response to vaccination as indexed by increases in inflammatory-responsive markers, a localized response, as indexed by elevated muscular pain at the site of vaccination, might also be present.

4.2. Effect of vaccination on mood

Despite the increases in inflammatory-responsive markers in this study, no effects of vaccination-induced inflammation on mood were observed. This finding is in contrast to a previous a study reporting that increases in IL-6, which were similar in magnitude to those found in the current study, were positively correlated with negative mood assessed by the POMS (Wright et al., 2005). Nevertheless, it should also be acknowledged that others have also failed to demonstrate increases in negative mood in response to the typhoid vaccine (Brydon et al., 2009; Strike et al., 2004). One of these studies revealed an absence of increased positive mood in response to a typhoid vaccination that was only observed during the saline control condition (Strike et al., 2004). However, similarly to the current study, the other study found no evidence for increased negative mood two hours after vaccination (Brydon et al., 2009). These somewhat inconsistent findings

indicate that typhoid vaccination may not be sufficiently provocative to induce negatively-valenced feelings. However, it is worth re-iterating that the extent of the increases in inflammatory-responsive markers in current study is similar in magnitude to that reported in previous studies using the same typhoid vaccine (Clapp et al., 2004; Hingorani et al., 2000; Padfield et al., 2010), and critically, are comparable to studies which have observed effects of vaccination on mood (Wright et al., 2005). It appears that there is more consistent evidence for increases in negative mood following endotoxin administration where the increases in inflammation are more substantial (Eisenberger et al., 2009, 2010; Reichenberg, 2001), particularly in comparison to vaccination (Bennermo et al., 2004; Hingorani et al., 2000; Kharbanda et al., 2002; Wright et al., 2005). Overall, these data suggest that a potent and substantial inflammatory response is required to induce increases in negative mood.

4.3. Effect of vaccination on cardiovascular function

With the exception of an overall trend for heart rate to decrease over time, which has been reported previously (Strike et al., 2004), no effects of vaccination on cardiovascular activity were observed. Previous studies have also found no effects of vaccination on blood pressure and heart rate (Chia et al., 2003; Padfield et al., 2010; Strike et al., 2004; Vlachopoulos et al., 2005), or cardiac output and total peripheral resistance (Vlachopoulos et al., 2005). Thus, acute vaccine-induced inflammation had no effect on the cardiovascular parameters assessed. This again is in contrast to endotoxin administration, which reliably results in altered cardiovascular function in both humans and animals (e.g., Suda et al., 2011; Suffredini et al., 1989; van Eijk et al., 2007). Further, there is evidence that chronic inflammation can affect cardiovascular activity, with elevations in inflammatory markers such as IL-6 and CRP inversely associated with heart rate variability, in both healthy populations and those with cardiovascular diseases (Haensel et al., 2008; Sajadieh et al., 2004; von Känel et al., 2011). Thus, it appears that a more substantial inflammatory stimulus may be necessary to induce impairment in cardiovascular function.

4.4. Study limitations and future directions

The current findings have potential implications regarding the vaccination of individuals with already elevated levels of inflammation, such as those at risk of cardiovascular disease, stroke, myocardial infarction or even an aging population. Although studies that have examined the risk of cardiovascular disease and myocardial infarction after vaccination have revealed no increased risk of myocardial infarction (Smeeth et al., 2004) and small reductions in the risk of incidence of cardiovascular death (Warren-Gash et al., 2009), these studies examined vaccines, such as influenza, that induce relatively weak inflammatory responses. The effects of a vaccine that induces a stronger inflammatory response, such as the typhoid vaccination, might warrant closer examination. Likewise, given the increased risk of myocardial infarction associated with infection (Meier et al., 1998), the potential contribution of vaccination to the triggering of myocardial infarction in an apparently healthy population seems worthy of further consideration. Indeed, given the known effects of acute inflammation on resting vascular function (Antoniades et al., 2011; Clapp et al., 2004; Hingorani et al., 2000), a time course-based examination of the effects of inflammation on vascular function also merits further investigation. It should also be acknowledged that there is a possibility for Type II error given the relatively small sample size in our study. Further, given the moderate effect sizes reported, the lack of effects of the vaccination on pain could be attributed to low statistical power (Cohen, 1992).

5. Conclusion

To our knowledge, this is the first time course study to examine the inflammatory effects of S. typhi vaccination, and its impact on mood, symptoms, and cardiovascular activity. It appears that the S. typhi vaccination induces a relatively mild inflammatory response, as evidenced by the doubling of IL-6 and TNF-α, which peaked at 6–8 h post vaccination. Elevations in the numbers of granulocytes were also demonstrated in the vaccination condition. CRP also increased following vaccination, with the largest increase, which represented a doubling compared to pre-vaccination levels, seen at 24 h post vaccination. There was a tendency for more physical symptoms and pain at the site of vaccination in the vaccination condition, but no effects of mild inflammation were observed on mood or any cardiovascular measure. Thus the S. typhi vaccination can be used to induce increases in inflammatory-responsive markers, without altering mood or cardiovascular activity.

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